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HEREDITY, CONSTITUTION AND TUBERCULOSIS AN EXPERIMENTAL STUDY

BY
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From the Henry Phipps Institute, University of Pennsylvania
Philadelphia, Pennsylvania

Edited by ESMOND R. LONG

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Edited by ESMOND R. LONG

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I. INHERITED NATIVE RESISTANCE AND NATURAL RESPIRATORY CONTAGION

INTRODUCTION

The purpose of the investigation here reported was to determine what is the rôle of the genetic constitution of rabbits in their resistance to tuberculosis and to identify the hereditary factors which control resistance or susceptibility to the disease.

While there are numerous studies (1) (2) demonstrating that, by selective breeding of animals, stocks can be obtained which are uniformly either highly resistant or highly susceptible to acute infectious diseases, through generation after generation of inbreeding, carefully controlled experimental investigations in the slowly progressive disease tuberculosis are but few (3). It would seem, however, that the very chronicity of this disease offers certain advantages in the analysis of the constitutional characters responsible for resistance or suceptibility. For tuberculosis is, as it were, a slow motion picture of acute infections. A deeper insight into the mechanism of natural resistance to tuberculosis may throw some light on the nature of resistance to infections in general.

The rôle of heredity in human tuberculosis still remains perplexing. There is no race of human beings which is completely immune to tuberculosis. However, the mortality and morbidity from tuberculosis vary widely in different races. Whether the differences are due to true genotypic inherited variations in native susceptibility and capacity for immunization is at present a widely debated question. The work of Bushnell (4) and particularly the tuberculosis observed by Borrel (5) among the Senegalese troops during the World War, which was characterized by massive caseation of the tracheobronchial lymph nodes, caseous pneumonia, extensive serositis and miliary tuberculosis, have led many investigators to explain the high mortality among these troops on the basis of a lack of protective childhood infection found so frequently in Europeans.

The mortality from tuberculosis in the Negro population of the United States is three to four times that of the white population. Pinner and Kasper (6) made careful comparative studies of the pathological charac-

ters of tuberculosis in the Negro and white man. They found that lymphogenous and haematogenous spread, caseous pneumonia, miliary tuberculosis, extensive caseation of lymph nodes and large nodular tuberculosis of internal viscera were much more common among Negroes than among whites. Similar observations were made by Everett (7). Progressive, so-called childhood type of tuberculosis with caseation of the tracheobronchial nodes was found in nearly one-half of the Negro adults. It is noteworthy that this type of tuberculosis in Negro adults frequently originated in the apex. Both groups of investigators emphasized the more rapidly fatal course of tuberculosis in the Negro.

That this difference in the disease in the Negro cannot be attributed to lack of childhood protective infections is indicated by the studies of Opie (8), who found that the incidence of calcified pulmonary lesions in the Negro is only slightly less than that of the white man. The incidence of tuberculous infection as indicated by the tuberculin reaction is greater among Negroes at all ages, not only in the East but also in the South (9). It is noteworthy that Aronson (10) found that Negroes are more sensitive to tuberculin, as indicated by the greater frequency of intense reactions and by the higher incidence of reactions to small amounts of tuberculin. Aronson (11) has also found that American Indians are more sensitive to tuberculin than the white population.

An extensive and illuminating study on tuberculosis in natives of the Rand in South Africa (12) revealed that, while more than 70 per cent of the natives react to 0.02 mg. of tuberculin on arrival, these natives often succumb to a rapidly fatal progressive tuberculosis characterized not only by massive caseation of the tracheobronchial nodes, but also by extensive caseation of the abdominal lymph nodes with massive caseating tuberculosis in the liver and spleen. In 50 per cent of these cases calcified primary lesions are found. The disease pursues the same rapidly fatal course whether the reaction to tuberculin is positive or negative on arrival. Furthermore, fully 50 per cent of healthy natives react to 1:1,000,000 dilution of tuberculin. These natives are therefore not "virgin soil" for tuberculosis. Yet if they remain in their kraals, the disease is often quiescent. It is only when they are exposed to the overwork, undernourishment and unaccustomed conditions in the mines that the disease extends so severely.

Furthermore, not only can great differences be shown in the type of tuberculosis in Negroes and whites, but conspicuous differences have been demonstrated among different groups of whites. Thus, Putnam

(13), in a study of 505 white families composed of Italians and Jews coming to the dispensary of the Henry Phipps Institute, found that among Italians the disease attacked a younger group and pursued a more severe, rapid and fatal course than among the Jewish families; yet analysis did not indicate that family size was a determining factor.

It is obvious from what has been reviewed that hereditary factors may play a rôle in determining the course of tuberculosis in different individuals or groups, and that apparently the more susceptible races manifest a higher degree of sensitivity to tuberculin. However, many of the differences observed may be ascribed to environmental variables.

In an effort to answer this question more directly, Diehl and Verschuer (14) studied tuberculosis in identical and fraternal twins. The former have the same genetic determinants; the latter are genetically different. Of 37 identical twins 26 behaved in a similar fashion toward tuberculosis. In the 11 remaining identical twins the tuberculosis pursued an unlike course. Thus there was 70 per cent correspondence between the genetic constitution and behavior toward tuberculosis. On the other hand, among 69 fraternal twins, i.e., genetically different twins, the tuberculosis was of the same nature in 17 and of a different character in 52 twins; the correspondence in this group was, therefore, only 25 per cent. From these studies the authors concluded that heredity plays a deciding rôle in tuberculosis. They assumed that the environmental conditions were the same for each member of any pair of twins irrespective whether they are identical or fraternal. However, as pointed out by Lange (15), the environmental factors were not sufficiently known and, in general, these were much more often the same for identical than for fraternal twins. The like behavior of identical twins could, therefore, be ascribed to the more frequent identical environmental conditions that prevailed among these.

The present resistance of certain racial groups or even individuals has often been attributed to the inheritance of immunity acquired by their forbears as a result of contact with the disease for several generations. It is clear, however, that this resistance can be explained not by postulating the inheritance of acquired immunity, which is contrary to one of the fundamental tenets of experimental biology, but on the basis of the elimination of susceptible individuals or groups before their reproduction and the perpetuation of the naturally resistant.

Plainly, therefore, studies in human beings remain difficult in interpretation. For this reason Wright and Lewis (3) studied the resistance

of guinea pig families that had been inbred for about fourteen generations by brother and sister mating. They found that, with subcutaneous injection of a standard dose of tubercle bacilli, the difference in longevity between the different families was as much as 40 per cent. It is evident from their studies, as critically analyzed by Hill (16), that one family of these guinea pigs is genetically endowed with a considerably greater resistance to tuberculosis than the others. In further studies Lewis and Loomis (17) correlated this increased resistance to tuberculosis of guinea pig families with their innate capacity to form antibodies. They found that the most resistant families produced a greater amount of haemolysins and agglutinins when exposed to the corresponding antigens.

Our own studies are essentially a continuation of the pioneer investigations begun in this Institute by Lewis and his associates. The impetus (18) for recommencing this work was the strange observation that, while vaccination of rabbits with living human type bacilli protected them against naturally acquired bovine tuberculosis for the first six months after the beginning of the exposure, at the end of a year some nonvaccinated rabbits failed to develop tuberculosis, whereas nearly all the vaccinated animals developed the disease. It was suggested by this experiment, that the natural innate resistance of some rabbits was greater than the acquired immunity of other individuals. The investigation has been continued since then for a period of more than seven years.

MATERIALS AND METHODS

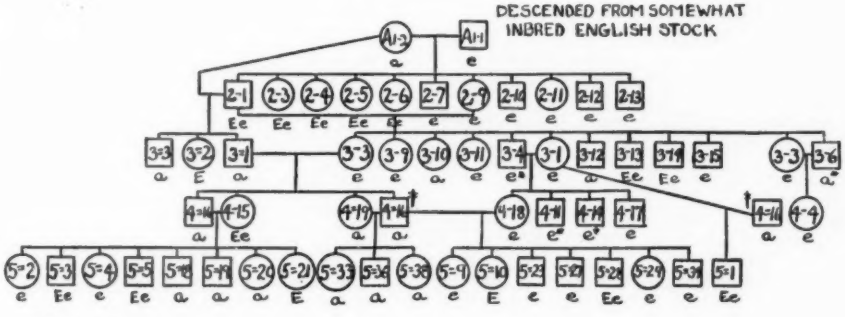
The Rabbit Families

Two groups of rabbits were obtained from widely separated parts of the country. One was kindly given to us by Dr. E. L. Stubbs of the School of Veterinary Medicine of the University of Pennsylvania; the other came from Michigan, the Swift stock originally obtained from Drs. Bull and Webster. The Pennsylvania group consisted of two families. The father, A1-1, of one family, A (chart 1), was a small English self black buck, descended from English stock, somewhat inbred. The self quality of English rabbits is represented by the duplex recessive genes *en en* (19). The mother was a large white rabbit of whose genetic constitution nothing was known. However, all the 23 young born to these two rabbits were colored. This proves that A1-1 had the color gene in duplex: *CC*. Furthermore, of these 23 young, 11 were English selves and 12 were exhibition English rabbits of the genetic constitution *En en*.

The proportion between these two types of young was about half and half. Therefore the white mother must have carried the genes *En en*, or the exhibition English color markings in suppressed form. Thus both the father and the mother of this family carried the English characters.

The genetic histories of all the rabbits of all the families reported in this and subsequent papers of this series are detailed in charts 1 to 8 inclusive. Rabbits of these families not reported in these studies are omitted from the charts to save space.

CHART 1
THE "A" FAMILY



* Angora.

‡ Rabbits with this sign are one and the same animal.

KEY TO ALL CHARTS

Only rabbits reported in this and subsequent papers of this series are included in these charts.

Designations for color distribution:

a = albino
h = himalaya
e = English self
E = Chaplin
Ee = Exhibition English
D = Exhibition Dutch
d = Dutch (light or low)

The father of the other family of this group, B (chart 2), was the same as the father of the A family, A1-1. The mother of the B family was an agouti self rabbit, descended from English stock, inbred to some extent. Thus it is clear that both families A and B carry the English characteristics on both sides of their forbears. It is significant that families A and B share a common ancestor. If resistance to tuberculosis is determined by hereditary characters, it is clear that families A and B may be ex-

pected to have certain behavior patterns toward the tubercle bacillus in common. This, as will be shown, has proved to be the case. The differences in the two families (and these were also striking) must be attributed to the white doe with the suppressed English determinants, who was the only different ancestor in the two families.

The other group of rabbits, the Swift stock, consisted of six families designated C, D, E, F, G, and H respectively. All of these families without exception (plotted in charts 3, 4, 5, 6, 7 and 8) had among their many ancestors one and the same Dutch rabbit, here named 1. All but the D family had in addition another common ancestor, here designated as 27. It is clear, therefore, that some common characters in all these six rabbit

CHART 2

THE "B" FAMILY

DESCENDED FROM SOMEWHAT INBRED ENGLISH STOCK

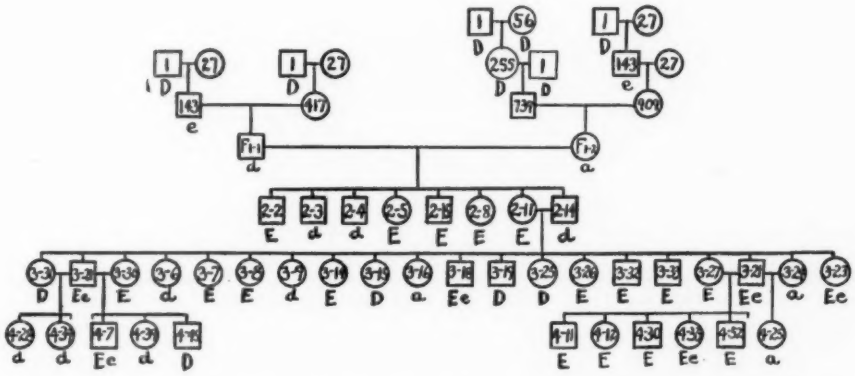
families are possible. Many additional rabbits, however, entered into the composition of these families.

As will be demonstrated in the course of this study, sharp and constant differences were apparent in the resistance to tuberculosis in these six families. Therefore rabbits other than 1 and 27 entering into the composition of each of the six families must be responsible for the differences observed between them, if resistance to tuberculosis is determined by the genetic constitution of individuals.

For the sake of simplicity let us first analyze the composition of the F family (chart 6). Rabbits 1 and 27 entered preponderantly into the composition of this family. There is only one other rabbit, 56, which occurred only once in the third removed generation on one side of the

forbears of the mother of this family, F1-2. From an examination of the chart it is evident that F1-1, the father of this family, was the first inbred generation of rabbits 1 and 27. F1-2, the mother of this family, was the product of considerable inbreeding of the same two rabbits. It will be shown that the F family is uniformly susceptible to tuberculosis through successive inbred generations. Therefore the preponderant ancestors of this family, rabbits 1 and 27, must both have carried the genetic constitution for susceptibility if resistance to tuberculosis is determined by the genetic constitution of an individual. Rabbit 56 was either susceptible to tuberculosis also, or its genes were not incorporated in the chromosomes of the parents of this family.

CHART 6
THE "F" FAMILY

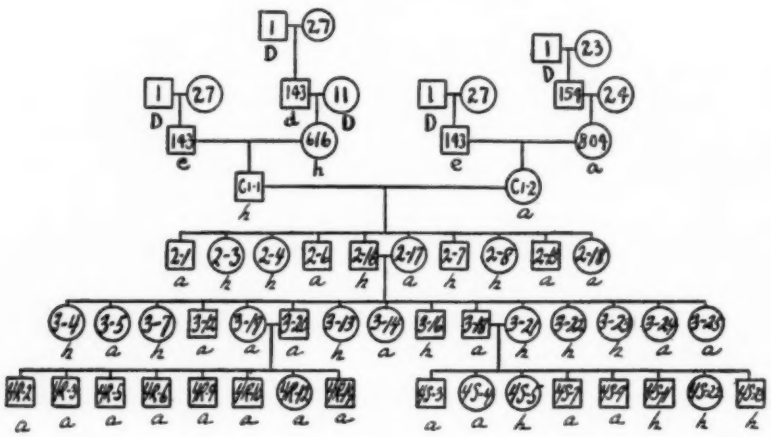


From chart 3 for family C it is evident that the same two rabbits 1 and 27 entered preponderantly into the ancestry of the parents of this family, C1-1 and C1-2, and that these two rabbits were the products of considerable inbreeding of rabbits 1 and 27. It is clear, therefore, that these two rabbits may be expected to play a dominant rôle in the composition of the C family. It will be clearly demonstrated in the course of this study that, in general, the C family is highly susceptible to tuberculosis, just like the F family, where the same two ancestors 1 and 27 play the preponderant rôle in their composition. Occasionally, however, rabbits in the C family were encountered which were relatively resistant to the infection. It is permissible to presume, therefore, that the aberrant behavior of these unusual C rabbits might have been due to determinants

for resistance carried by one or more of the remaining forbears of this family, namely, 11, 23 and 24. A glance at the chart shows, however, that these rabbits entered far less frequently into the forebears of this family.

Chart 8 of the H family shows that rabbits 1 and 27 were only 2 of the 8 ancestors of this family; the other contributors to the genetic constitution of this family were rabbits 21, 67, 2, 5, 62 and 24. By direct experiment it was found that the immediate offsprings of H1-1 ♂ and H1-2 ♀, the parents of this family, were quite susceptible to tuberculosis. These rabbits are referred to here as H2, or second inbred generation rabbits. The only common ancestors of the H2 rabbits and of the families F and

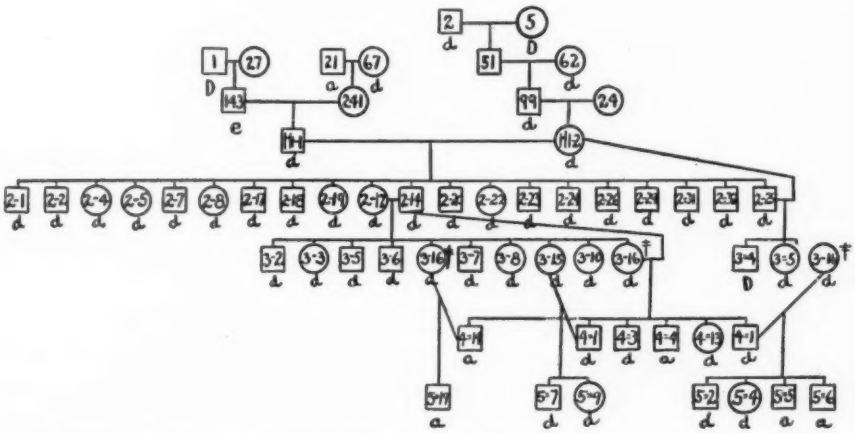
CHART 3
THE "C" FAMILY



C were again rabbits 1 and 27. Nothing is known about the remaining ancestors of this family. Whether these two rabbits were responsible for the relative susceptibility of the H2 rabbits is not certain. It is, however, a possibility. The descendants of the H2 rabbits were definitely more resistant than their parents. It is permissible to presume that one or more of the remaining ancestors of the H family, rabbits 21, 67, 2, 5, 62 and 24, carried determinants for resistance to tuberculosis. For it is possible that in the composition of the later inbred generations of these rabbits the factors for susceptibility derived from rabbits 1 and 27 might be diminished, as the remaining ancestors definitely outnumbered these presumably susceptible animals.

An examination of the pedigree of the D family (chart 4) shows that the parents of this family, designated as D1-1 ♂ and D1-2 ♀ had some common ancestors; therefore the offspring of these two rabbits, the D2 rabbits, were inbred to some extent. The rabbits entering into the composition of the D family were 1, 11, 21, 2, 26 and 53. Rabbit 1 was common to all the families of the Swift group and was the only one in the D family of whom it can be said, according to the preceding analysis, that it carried determinants for susceptibility. Rabbit 11 occurred in the forbears of the C family, where analysis lists it as possibly resistant. Similarly rabbits 21 and 2 are found in the ascendency of the H family and

CHART 8
THE "H" FAMILY



‡ Rabbits with this sign are one and the same animal.

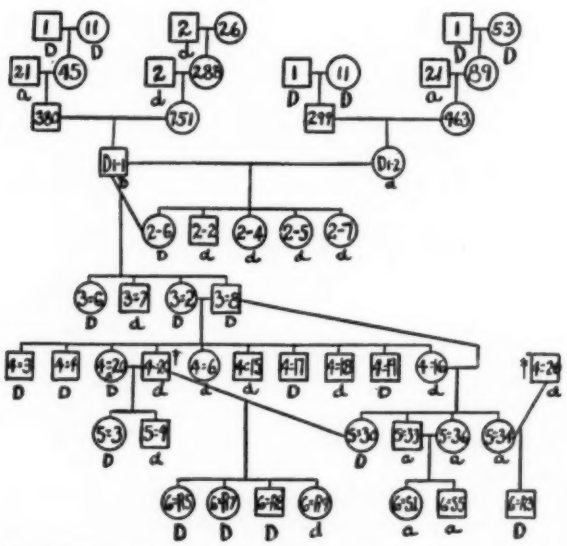
are there considered as possibly resistant stock. It will be shown later that the D family is definitely resistant to tuberculosis. It is possible, therefore, that one or more of these three rabbits carried determinants for resistance. Nothing is known of the remaining ancestors of this family, 26 and 53.

For the remaining two families of the Swift group, G and E, (charts 7 and 5), not enough experimental data are available to place them clearly in a particular category of susceptibility or resistance. Nevertheless the little information that is at hand is not at variance with the presumed characters of the ancestors of these families. Rabbits 1, 27, 2 and 24 were common to the G and H families, and 1, 27 and 11 were common

to the G and C families. This would place the G family in an intermediate position between the H and C families. This relative position of the G's is in agreement with the small number of observations available for this family.

Similar statements could be made of the E family. E1-1, the father of the E family, was a litter-mate of H1-1, the father of the H family. Of the 5 ancestors of the E family, 4 rabbits, 1, 27, 21 and 67, were common to both E and H families. Therefore, one could expect certain similarities in the behavior of these families. The limited data available for this family are in accord with this analysis.

CHART 4
THE "D" FAMILY



‡ Rabbits with this sign are one and the same animal.

Thus an analysis of the admittedly meagre data on the forbears of all these families is in harmony with the fundamental conclusion of this work, namely, that, *ceteris paribus*, resistance to tuberculosis is determined by inherited constitutional factors. The experimental observations upon which this conclusion is based can now be presented in detail.

Breeding and Rearing of Families

The following scheme of identification of all the rabbits was used in this study: each doe and buck with their young was considered a family. The

parents were tatooed on the inside of the ear with the letters A, B, C, D, E, F, G, H. The father of each family was labelled A1-1, B1-1, C1-1, etc. The mother of each family was labelled A1-2, B1-2, C1-2, etc. The young in each family were labelled A2-1, A2-2, A2-3, etc., B2-1, B2-2, B2-3, etc. Since it was shown above that most of the parents of these families had been inbred to some extent before they came to our laboratories, these parents were arbitrarily designated as the first inbred generation. Their young were, therefore, called the second inbred generation. After that generation the families

CHART 7

THE "G" FAMILY

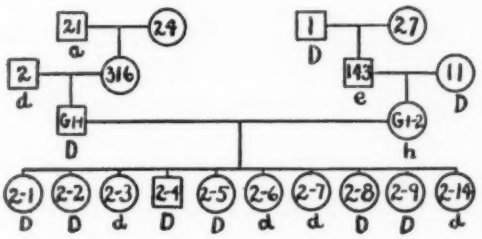
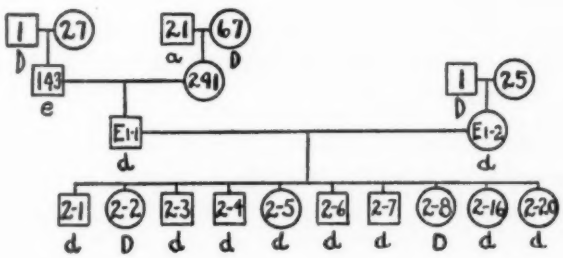


CHART 5

THE "E" FAMILY



have been continued up to the present time by either brother and sister or parent and offspring mating without outbreeding to any other family. It was not always possible to breed brother and sister. Young resulting from parent-offspring matings were designated with a generation number following that of the most inbred parent. Thus offspring of the backcross A1- and A2 were called A3 = with the double dash; whereas offspring of brother and sister A2 and A2 were called A3- with a single dash. The double dash rabbits therefore belong only partly to the designated inbred generation, whereas single dash rabbits belong fully to the indicated inbred generation.

In breeding these families the mothers were permitted to suckle their young for a period of five to six weeks. At this time the young were weaned, tatooed, separated and placed in individual cages. All the breeding animals and their young were fed Pratt's pellets or Purina chow, supplemented by green vegetables twice weekly. Wheat germ was added to the feed of the breeders. The animal caretaker of these breeding animals did not handle any tuberculous animals. The latter were kept in a different part of the Henry Phipps Institute.

Methods of Testing Resistance to Tuberculosis

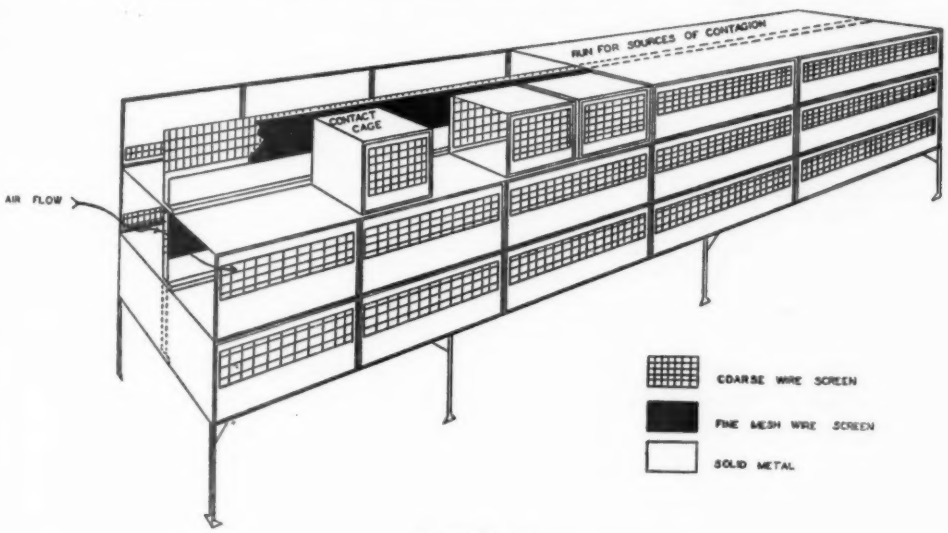
Each successive generation was tested for its resistance to tuberculosis by direct inoculation of standard quantities of tubercle bacilli intravenously or intracutaneously. The second generation of all the families was also tested for its resistance to tuberculosis by the instillation of the same quantity of tubercle bacilli into the conjunctival sac. All these procedures of artificial infection, however, proved far less informative on the behavior of the several families toward tuberculosis than the method of natural respiratory contagion.

This method of infection, consuming, as it did, very long periods of time (about a year and a half for each test) and involving, as it did, great difficulties in keeping animals crowded together for very long periods, nevertheless threw a searching light on the varying resistance of these families to tuberculosis, and, besides, pointed to the probable mechanism of this resistance in the several families. Furthermore, this method of infection closely simulated the mode of infection as it occurs in man and reproduced in its essential characteristics the varying types of the disease as it is seen in human beings.

In general, the experiments were conducted as follows: Four rabbits of a given generation of each family, individuals that had never been in contact with tuberculosis, were simultaneously placed on one side of a large cage divided in the middle by a fine wire mesh screen. These rabbits were exposed to rabbits infected intravenously with 0.000,1 to 0.001 mg. of highly virulent bovine type tubercle bacilli of the Ravenel strain. The disease produced in the latter rabbits, the sources of contagion, was chronic as a rule. The main source of tubercle bacilli for the contacts on the opposite side of the screen was the urine shed by these rabbits. This was shown in tests of the urine removed from the bladders of these rabbits during their life. Viable bacilli were demonstrated in the urine in 40 per cent of such rabbits on one or more occasions during the course of their infection. As is generally known, slowly progressive renal tuberculosis in the rabbit is characterized by tubular spread toward the

medulla from the original cortical lesions resulting from intravenous inoculation. Soon the tuberculosis ulcerates into the pelvis of the kidney and the urine becomes contaminated with tubercle bacilli. Other, though less frequent, sources of bacilli from such rabbits were the faeces and the nasal discharges. Tubercle bacilli could be demonstrated in these excreta of infected rabbits from time to time.

The cages opened into the animal room from below on the side where the sources of contagion were kept, and from above where the contacts were confined. The air, warmed by the sources of contagion, rose, crossed the screen and passed into the room from that portion of the cage where the contacts were stationed. Repeated tests with *Bacillus prodigiosus* demonstrated that



TEXT-FIG. 1

bacteria placed on the floor of the sources of contagion could be isolated on agar plates placed on the contact side of the cage. In the seven years during which the study was conducted the cages used in these experiments varied somewhat in construction, but the principle of all of them was the one just given. The cage as finally perfected, and as used in the last two years, is pictured in text-figure 1. In this manifold, 30 contacts were placed in individual cages. This procedure largely obviated cross contagion of tuberculosis among the contacts. It also helped greatly in keeping the animals alive over long periods of time. By this means, loss of contacts due to intercurrent diseases was reduced to a minimum. Furthermore, in order to equalize as nearly as possible the intensity of contagion to which each individual rabbit was exposed, these cages were constantly shifted during the entire duration of the exposure. This was

accomplished by moving each cage every day to a new position, so that in the course of thirty days each contact was in all of the thirty positions.

On the other side of the centrally placed screens, 10 infected rabbits were placed in each of the three runs. These 10 rabbits served as sources of contagion for the 10 contacts in the individual cages on the opposite side of the screen. The sources of contagion were kept constant throughout the duration of the experiment by replacing the dying rabbits by similarly infected animals.

Every two to four weeks following the beginning of exposure, each contact was tested with 0.1 cc. of 1:10 dilution of Old Tuberculin, prepared from the same microörganism, the Ravenel strain, which was used to infect the sources of contagion. Every month the lungs of these rabbits were X-rayed, since the disease acquired by these contacts in all but rare instances originated in the lung. Frequently the tuberculin reaction became definitely positive before any clear roentgenological evidence of tuberculosis developed. In other instances tuberculin hypersensitivity failed to develop to any considerable extent. The time of onset of the disease in these contacts had to be estimated on the basis of roentgenological evidence alone. Most frequently, however, the two diagnostic methods complemented each other, and it was on the evidence of both of these that the time of onset of the disease in any contact was decided. The exposure was not discontinued after the onset of tuberculosis. On the contrary, the rabbit remained in the manifold until its death. It might have been more desirable to cease exposure with the acquisition of tuberculosis. However, these experiments were of such long duration, that at times as many as eleven months elapsed before some rabbits began to show unmistakable evidence of tuberculosis, and since the greatest number of rabbits that could be exposed at one time was only 30, it was thought more advisable to continue the exposure until the death of the animal. In this manner, it is believed, the constancy of exposure of all the representatives of the different families was more uniform than if exposure ceased at the onset of the disease.

When the rabbits died, a careful autopsy was performed. An effort was made to reconstruct as far as possible, on the basis of the pathological findings, the site of onset of the disease, the progression of the primary lesion and the spread of the tuberculosis to the rest of the body. In each instance the diagnosis of tuberculosis was confirmed by histological demonstration of typical tuberculous lesions and typical acid-fast bacilli. Guinea pig inoculation was practiced in case of doubt. Histological analysis of the various lesions helped greatly in the understanding of the type of tuberculosis developed by each member of each family. In this manner successively inbred generations of these families were tested at the same time.

Only the most significant observations in all these families are presented in this paper.

EXPERIMENTAL RESULTS

The A Family

In table 1 are recorded the histories of all the rabbits exposed for a minimum of five months from the second through the fifth inbred generations in the A family. In order to save space those exposed for less than this period, that failed to develop tuberculosis, are omitted from this table. The same procedure has been followed in the case of all the other families. All rabbits that developed tuberculosis in this and all the other families, are included, irrespective of the duration of exposure. It will be noted that a total of 16 rabbits, males and females in equal numbers, were exposed for a period varying between nine and nineteen months. The pedigree of each of these rabbits can be found in chart 1. The ages of these rabbits at the beginning of exposure ranged from 2.5 to twelve months. The median age was 3.5 months.

Of the 16 rabbits exposed, 10, or 62 per cent, acquired tuberculosis.

In a preliminary paper on this investigation (20) it was reported that resistance to tuberculosis was inversely related to the spread of India ink injected intracutaneously. The greater the spread of India ink in the skin on the sixtieth day after a cutaneous infection with virulent tubercle bacilli, the less was the resistance of that family, as measured by the survival period after such a standard infection. Prior to the beginning of exposure of the later inbred generations of these families, the spread of 0.5 cc. of autoclaved 1:5 dilution of India ink in saline was determined one day after intracutaneous injection. The area of spread is given in square mm. It was arbitrarily obtained by multiplying the two largest diameters of the stained skin site at right angles to each other. The spread of India ink was measured in 5 rabbits, 2 females and 3 males, of the fifth inbred generation of this family prior to the beginning of exposure. It will be noted in column 2 that this spread varied from 238 to 720 square mm., the average being 546.

In the next column is recorded the pretuberculous period or the total duration of exposure. By the pretuberculous period is meant the number of months that elapsed from the beginning of exposure to the onset of tuberculosis, determined as described above. This interval varied greatly from rabbit to rabbit and averaged 5.6 months.

In the fourth column is recorded the maximum sensitivity to tuberculin attained by each rabbit during the entire period of the exposure. It is given in numerical form. It represents the volume of inflammation that resulted two days after the intracutaneous injection of 0.1 cc. of a

TABLE I

Naturally acquired respiratory tuberculosis in rabbit family A

RABBIT NUMBER, FAMILY, GENERATION, SEX AND AGE AT THE BEGINNING OF EXPOSURE	INDIA INK SPREAD BEFORE EXPOSURE IN SQ. MM.	PRE-TUBERCULOUS PERIOD IN MONTHS, OR DURATION OF EXPOSURE	MAXIMUM VOLUME OF TUBERCULIN REACTION IN CMM.	CHARACTER OF PRIMARY LESION	LESIONS IN DRAINING TRACHEOBRONCHIAL LYMPH NODES	CAVITY FORMATION	CANALICULAR SPREAD	HAEMATOGENOUS AND LYMPHOGENOUS SPREAD	NUMBER AND EXTENT OF EXTRA-PULMONARY VASCULAR METASTASES	DURATION OF DISEASE IN MONTHS	ANATOMICAL TYPE OF DISEASE
A2-11 ♀ 3 mos.	—	5	149	Firmly walled off cavities	0	Numerous	Tbc. of lungs and intestines	Slight tbc. in kidneys and mammary glands; slight pleural tbc.	3 slight	7.5	Ulcerative pulmonary phthisis of "reinfection" type
A2-10 ♂ 3.0	—	8	226	?	0	Numerous	Tbc. of lungs, intestines and testis	Slight tbc. in kidneys	1 slight	6.5	Ulcerative pulmonary phthisis of "reinfection" type
A2-4 ♀ 3.0	—	9	156	Large firmly walled off cavity	0	Numerous	Tbc. of lungs and intestines	Chronic ulcerative renal tbc.; moderate pleural tbc. and tbc. of ovary and mammary gland	4 moderate	7.2	Ulcerative pulmonary phthisis of "reinfection" type
A2-6 ♀ 3.0	—	9	19	Large interlobular cavity	0	Several	Tbc. of lungs, intestines, larynx and epiglotis with caseation of cervical nodes	Slight renal and pleural tbc. Small discrete noncaseated pulmonary tubercles	2 slight	9.0	Ulcerative pulmonary phthisis of "reinfection" type
A3-2 ♀ 3.5	—	19	0								Killed; no tbc.
A3-3 ♂ 3.5	—	3.2	22	Large cavity	0	One	Microscopic evidence of bronchogenic spread	Nodular pulmonary tbc.; single renal lesion; slight pleural tbc. and tbc. of one joint	3 slight	11.5	Chronic tbc. of "reinfection" type
A3-13 ♂ 2.5	—	19	0								Killed; no tbc.

A3-15 ♂ 2.5	—	5	400	?	0	Numerous	Tbc. of lungs, intestines and testis	Slight renal and pleural tbc.	2 slight	7.0	Ulcerative pulmonary phthisis of "reinfection" type
A3-14 ♂ 2.5	—	5.6	0	?	0	None	Tbc. of intestines with peritonitis and caseation of mesenteric nodes; tbc. of testis	Slight cortico-medullary renal tbc.; tbc. of iris; slight tbc. of pleura and adrenal; fibrocaseous pneumonia	4 slight	10	Chronic fibro-caseous pneumonia of "reinfection" type
A3-10 ♀ 2.5	—	6.4	0								Died; no tbc.
A4-4 ♀ 11.0	—	17.0	+								Killed; no tbc.
A5-2 ♀ 12.0	546	8	+								Died; no tbc.
A5-3 ♂ 12.0	238	3.3	86	Single thin walled cavity	0	One	Tbc. of epididymis, prostate and testis	Miliary noncaseated pulmonary tubercles; minimal pleural tbc.; slight cortico-medullary renal tbc.	2 slight	12.7	Chronic tbc. of "reinfection" type
A5-4 ♀ 12.0	580	5	0								Died; no tbc.
A5-21 ♂ 8.5	646	3	2160	?	0	None	Tbc. of intestines with peritonitis; tbc. of epiglottis and epididymis	Moderate pleural tbc.; minimal renal tbc.; discrete nodular, caseous, pulmonary tbc.	2 slight	11	Chronic tbc. of "reinfection" type
A5-23 ♂ 7.0	720	4.5	989	Single thin walled cavity	0	One	None	Massive pleural tbc. with cavity; slight tbc. of iris and thyroid; moderate renal tbc. and noncaseated pulmonary nodules	4 moderate	10.5	Chronic ulcerative tbc., chiefly pleural, of "reinfection" type

10 per cent dilution of the Old Tuberculin described above. This volume was obtained as follows: The two largest diameters of the lesion at right angles to one another were measured. The thickness of the lesion was determined by applying calipers closely to the folded skin and subtracting therefrom the thickness of a fold of unaffected skin in immediate proximity. The volume of this lesion was calculated according to the following formula for the volume of a segment of a spherical polygon (21):

$$1/6\ \pi\ h\ (h^2 + 3\ a^2)$$

in which h is half the thickness of the lesion as measured above, and a is half the sum of the two largest radii.

It will be seen that in general the tuberculin sensitivity developed by the members of the A family that acquired the disease was not very pronounced. This will be apparent later when these reactions are compared with those of other families. Rabbits A 5=21 and A 5=23 developed extensive but very atypical reactions to tuberculin. These were vaguely defined, oedematous swellings, with little redness or induration, and resembled closely a mild, local, anaphylactic type of skin reaction. The maximum volume of the tuberculin reactions of the remaining rabbits of this family varied between 19 and 400 cmm. The mean volume of these reactions, exclusive of A5=21 and A5=23, was 132 cmm. It will be noted that rabbits A4-4 and A5=2 showed clearly positive tuberculin reactions; yet, at autopsy, no tuberculosis could be found in them.

The primary pulmonary focus, where such could be identified with some certainty, was, in the majority of instances, a single thick or thin-walled cavity (figure 2). From these primary pulmonary foci two modes of spread to the remainder of the lung and to the rest of the body are possible: vascular and canalicular. The former includes both lymph and blood vessels; the latter is through the alveolar passages and bronchi. In not a single instance in all of the 10 rabbits that acquired the disease were there gross tuberculous changes in the draining tracheobronchial lymph nodes (figures 1 and 2).

The absence of tuberculosis from the draining tracheobronchial lymph nodes in all these rabbits makes the disease acquired by the A family analogous to the reinfection type of tuberculosis. This term is here used in contrast with the designation of primary progressive tuberculosis on the basis of the fundamental distinction drawn by Robert Koch of the behavior of the lymph nodes draining the site of a primary inoculation and that of a reinoculation. There is no implication thereby that the

given disease is actually preceded by a first infection. While the hilum nodes were never affected grossly in any of these rabbits, pleural tuberculosis involving the costal, mediastinal and diaphragmatic portions was quite frequent. This was usually very slight; in one instance, however, the pleural disease was extensive. This pleural tuberculosis begins as a slight thickening of the membrane along the course of the lymphatics surrounding the intercostal vessels.

In nearly all these rabbits there was evidence of dissemination of the disease by way of the blood stream, as indicated by some renal involvement in each instance. However, this tuberculosis was slight indeed, and in many instances nothing but scars and small pittings on the cortical surface of the kidneys remained; rarely a mammary gland, an ovary, an adrenal, the thyroid, a joint or the iris was the seat of nonprogressive, inconspicuous lesions. As a rule, even the tubercle bacilli that were filtered out from the blood in the lungs did not prosper. This is illustrated in rabbits A2-6 (figure 2) and A5=3. In both instances there was one cavity and in the remainder of both lungs there were small discrete, round or stellate, subpleural tubercles uniformly distributed over both lungs. It is clear that haematogenous dissemination of the disease, while occurring regularly, played but a minor rôle in the progression of the disease and its fatal outcome in the A family. This is clearly seen in the tenth column, where the number and extent of the extrapulmonary vascular metastases are recorded.

Of far greater importance was the rôle of canalicular spread and extension by continuity in the fate of the tuberculosis in this family. In a large proportion of cases the disease was chiefly limited to the lungs, the portal of entry of the bacillus. Frequently the entire lung was replaced by numerous, anastomosing, honeycombed cavities (figure 1). The disease then resembled most closely ulcerative pulmonary phthisis as seen in adult human beings, especially in the white races. Canalicular spread also accounted for the frequent, extensive lesions encountered in the intestines, larynx and epiglottis (figure 2). Similarly haematogenous tuberculosis rarely progressed to any degree in the kidneys of these rabbits, but extensive tuberculosis of the epididymis, testis and prostate was often encountered. This obviously resulted from canalicular spread of bacilli from lesions ulcerating into the medullary tubules and pelvis of the kidney.

The duration of the disease in these 10 rabbits varied from 6.5 to 12.7 months with an average illness of 9.3 months. As will be seen, this is by far the most chronic disease in all the families studied.

It will be noted that no significant difference in any of these criteria of resistance and mode of development of the disease was seen in any of the second, third, fourth and fifth inbred generations. All these generations behaved toward tuberculosis in essentially the same manner.

Microscopic examination of tissue in the lungs from these rabbits confirmed the gross observations. Usually, cavities with thick walls composed of many layers of intact collagenous connective tissue (figure 14), often surrounding a zone of intact epithelioid cells, were seen. Softening, liquefaction and occasional foci of calcification were encountered within the encapsulated areas. Tubercle bacilli were found, at times in tremendous numbers, in the foci of early softening (figure 17). Rarely, blood vessels became enmeshed within this softening material. Opportunity for blood invasion was apparent. Caseous tissue, unaffected by the softening process, rarely contained tubercle bacilli (figure 17). Necrotic open vessels within the caseous foci were rarely seen. Perivascular or subpleural epithelioid cell tubercles in the lungs showed little caseation and rare bacilli. A definite tendency to resolve completely or to heal by cicatrization with resulting atelectatic areas was noted (figure 16). Similar resolving miliary tubercles were seen occasionally in the iris and ovary. The haematogenous tuberculosis in the kidney definitely healed in the cortex by fibrosis; however, tubular spread at

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FIG. 1. (Upper left.) Organs of rabbit A3-15, of the third inbred generation of the A family. Pretuberculous period, five months. Duration of disease, seven months. Massive ulcerative, pulmonary phthisis analogous to the "reinfection type." Draining tracheobronchial nodes normal. Healed renal tuberculosis and slight lesions in epididymis of tubular origin.

FIG. 2. (Upper right.) Organs of rabbit A2-6. Pretuberculous period, nine months. Duration of disease, nine months. Single, large, well encapsulated cavity in the right lung. Draining tracheobronchial nodes normal. Tuberculosis analogous to the "reinfection type," with tubular spread to the entodermal tract, including larynx and intestines, with ulceration in the colon. Mesenteric nodes normal. Kidneys normal. Nonprogressive, uniformly distributed, nodular tubercles in both lungs, of haematogenous origin.

FIG. 3. (Lower left.) Organs of rabbit F4-33. Pretuberculous period, six months. Duration of disease, 3.3 months. Large, completely caseous primary focus in middle of left lung without softening. Extensive enlargement and massive caseation of homolateral draining tracheobronchial nodes. Corresponding structures on right side not involved. Large nodular caseous tubercles of haematogenous origin in both lungs. Primary, rapidly progressive, generalized tuberculosis with extensive disease in the kidneys, pleura and knee joint.

FIG. 4. (Lower right.) Organs of rabbit F2-3. Pretuberculous period, six months. Duration of disease, five months. Confluent, nodular, caseous pneumonia with extensive caseation of draining tracheobronchial nodes. Primary progressive generalized tuberculosis with extensive disease in the kidneys and in one eye and involvement of pleura and trachea.

TABLE 2

Naturally acquired respiratory tuberculosis in rabbit family F

RABBIT NUMBER, FAMILY, GENERATION, SEX AND AGE AT THE BEGINNING OF EXPOSURE	INDIA INK SPREAD BEFORE EXPOSURE IN SQ. MM.	PRETUBERCULOUS PERIOD IN MONTHS, OR DURATION OF EXPOSURE	MAXIMUM VOLUME OF TUBERCULIN REACTION IN CMM.	CHARACTER OF PRIMARY LESION	LESIONS IN DRAINING TRACHEOBRONCHIAL LYMPH NODES	CAVITY FORMATION	CANALICULAR SPREAD	HAEMATOGENOUS AND LYMPHOGENOUS SPREAD	NUMBER AND EXTENT OF EXTRAPULMONARY VASCULAR METASTASES	DURATION OF DISEASE IN MONTHS	ANATOMICAL TYPE OF DISEASE
F2-8 ♀ 6.0 mos.	—	7	136	?	++++	None (softening)	Intestinal tbc.	Nodular pulmonary caseous tbc.; massive progressive renal tbc.; massive pleural tbc.; caseous tbc. of cervical nodes	4 massive	5	Primary progressive generalized tbc. with caseous pneumonia
F2-4 ♂ 5.0	—	7.5									Killed; no tbc.
F2-2 ♂ 5.0	—	9.5									Died; no tbc.
F2-5 ♀ 5.0	—	5.0	16	?	+++	None (softening)	Ulcerative intestinal tbc. with peritonitis	Nodular pulmonary caseous tbc.; moderate pleural tbc.; progressive renal tbc.	3 extensive	5.5	Primary progressive generalized tbc. with caseous pneumonia
F2-3 ♂ 5.0	—	6.0	165	?	++++	None (softening)	Intestinal tbc.	Nodular pulmonary caseous tbc.; progressive renal tbc.; extensive pleural tbc.; massive tbc. of one eye; tbc. of submaxillary node, thyroid gland and cricoid cartilage	7 massive	5.0	Primary progressive generalized tbc. with caseous pneumonia

FIGS. 1–4

F2-15 ♂ 4.5	—	3	324	Caseous pneumonic focus	++++	None	Gastro-intestinal tbc. with peritonitis	Nodular pulmonary caseous tbc.; massive renal tbc.; extensive pleural tbc.; tbc. of thyroid and left adrenal	5 massive	6.2	Primary progressive generalized tbc. with caseous pneumonia
F3-6 ♀ 11.0	—	17									Killed; no tbc.
F3-7 ♀ 11.0	—	11	1088	?	+++	None	Slight intestinal tbc. with caseation of mesenteric nodes	Nodular pulmonary caseous tbc.; extensive pleural tbc.; extensive progressive renal tbc.; tbc. of spleen, adrenals, bone marrow, general lymphatic system, uterus and conjunctiva	9 extensive	3.5	Primary progressive generalized tbc. with caseous pneumonia
F3-8 ♀ 5.3	—	10	388	Caseous pneumonic focus	++++	None	Slight intestinal tbc.	Nodular pulmonary caseous tbc.; massive pleural tbc.; progressive tbc. of the only kidney present; tbc. of lymphatic system	4 massive	6	Primary progressive generalized tbc. with caseous pneumonia and massive pleural tbc.
F3-9 ♀ 5.3	—	6.5	135	Caseous pneumonic focus	+++	None	Slight intestinal tbc.	Nodular pulmonary caseous tbc.; extensive progressive renal tbc.; extensive pleural tbc.; tbc. of adrenals and ovaries	5 extensive	8	Primary progressive generalized tbc. with caseous pneumonia
F4-25 ♀ 6.0	980	2	561	Caseous pneumonic focus	++++	None	Intestinal tbc. with discrete caseous foci in mesenteric nodes	Nodular pulmonary caseous tbc.; extensive pleural tbc.; progressive renal tbc.; tbc. of muscles, spleen, bone marrow, knee joint, parietal bone and brain cortex	9 extensive	4	Primary progressive generalized tbc. with caseous pneumonia

TABLE 2—*Concluded*

RABBIT NUMBER, FAMILY, GENERATION, SEX AND AGE AT THE BEGINNING OF EXPOSURE	INDIA INK SPREAD BEFORE EXPOSURE IN SQ. MM.	PRETUBERCULOUS PERIOD IN MONTHS, OR DURATION OF EXPOSURE	MAXIMUM VOLUME OF TUBERCULIN REACTION IN CMM.	CHARACTER OF PRIMARY LESION	LESIONS IN DRAINING TRACHEOBRONCHIAL LYMPH NODES	CAVITY FORMATION	CANALICULAR SPREAD	HAEMATOGENOUS AND LYMPHOGENOUS SPREAD	NUMBER AND EXTENT OF EXTRAPULMONARY VASCULAR METASTASES	DURATION OF DISEASE IN MONTHS	ANATOMICAL TYPE OF DISEASE
F4-12 ♀ 9.0	957	—	?	—	—	—	—	—	—	—	Unilateral snuffles pulmonary abscesses with acid fast bacilli
F4-11 ♂ 9.0	780	11.5	420	Two caseous pneumonic foci	++++	None	Tuberculous ulceration of trachea and intestinal tbc.	Nodular pulmonary caseous tbc.; extensive pleural tbc.; progressive renal tbc.	3 extensive	4	Primary progressive generalized tbc. with caseous pneumonia
F4-30 ♂ 4.0	460	2.5	352	Caseous pneumonic focus	++++	None (softening)	Tbc. of trachea and of intestines	Nodular pulmonary caseous tbc.; extensive pleural tbc.; progressive renal tbc.	3 massive	3	Primary progressive generalized tbc. and caseous pneumonia
F4-33 ♀ 4.0	960	6.0	364	Caseous pneumonic focus	++++	None	Tbc. of trachea with ulceration; intestinal tbc. and involvement of mesenteric nodes	Nodular pulmonary caseous tbc.; massive pleural tbc.; extensive progressive renal tbc.; tbc. of one joint and of skull bones	5 massive	3.3	Primary progressive generalized tbc. with caseous pneumonia

times extended the disease to the testis and other organs of the urogenital system. Areas of intra-alveolar collections of intact epithelioid cells, obviously derived from canalicular spread from the cavities, also rarely contained tubercle bacilli (figure 16). The tracheobronchial nodes were entirely free from microscopic tuberculous changes, except in the nodes of one single rabbit, in which a few minute epithelioid cell collections without caseation and without bacilli were seen. It is clear, therefore, that the tubercle bacilli were everywhere largely destroyed by the epithelioid cells in this family. They multiplied only in the foci of softening. By tubular spread the disease extended to other portions of the body. Haematogenous and lymphogenous dissemination of bacilli usually led to healing or regressive tubercles irrespective of the organ focalized.

The F Family

In sharp contrast to the behavior of the A family toward tuberculosis is the response of the F family to this infection. The essential data for the individuals of this family are recorded in table 2. The pedigrees of the rabbits studied are given in chart 6.

A total of 15 rabbits from the second, third and fourth inbred generations were exposed simultaneously with members of corresponding inbred generations of the other families. Six of these were males and 9 were females. Their age ranged between 4 and 11 months with a median of 5.3.

The spread of India ink in the skin of two males and two females of the fourth inbred generation was determined prior to the beginning of exposure. This ranged from 460 to 960 square mm., with an average spread of 827. This spread is clearly greater than that in the A family where the average spread was 546.

Of the 15 rabbits exposed for a period of two to seventeen months 12 or 80 per cent acquired tuberculosis.

The pretuberculous period varied from two to 11.5 months with an average of 6.4. No significant difference between the A and F families was seen in this regard. However, as can be seen clearly in column 4, the maximum tuberculin sensitivity developed by the rabbits of this family that acquired tuberculosis was usually pronounced. In two instances only was the volume of inflammation in response to Old Tuberculin less than 100 cmm. In the remaining 10 contacts this ranged from 135 to 1088 cmm. of typical, sharply circumscribed, reddened induration. The average maximum size of the tuberculin reaction developed

by all the 12 rabbits was 332 cmm., which is to be contrasted with an average of 132 cmm. developed by the rabbits of the A family.

The lesion at the portal of entry, wherever this could be ascertained, was a single or a double, rapidly progressive, caseous pneumonic focus, without encapsulation, but with beginning central softening in a minority of instances (figure 3). With the single exception of F4-12, considered below as a case of questionable tuberculosis, all the remaining 11 rabbits showed extensive to massive caseation of the tracheobronchial lymph nodes draining these fulminating primary pulmonary foci (figures 3 and 4).

Rabbit F4-12 showed a most unusual disease. The right lung was massively enlarged and filled with large encapsulated abscesses. Macroscopically and microscopically there was no structure anywhere in the body resembling in any way a tuberculous process. The opposite lung and all the other organs were entirely free of any tuberculous lesions. Rare acid-fast bacilli, however, were found in the pus of these abscesses from the right lung, together with non-acid-fast bacteria resembling *B. lepisepticus*. Whether these were true tubercle bacilli was unfortunately not determined by animal inoculation. In any case this disease, if it was tuberculosis, was *sui generis*, for in the thousands of tuberculous rabbits examined by the present and other workers, no such disease was ever encountered. Possibly tubercle bacilli were engrafted in the pus of the snuffles pneumonia abscesses. In any event no true tuberculosis resulted; hence this rabbit is not considered as a representative of the response of the F family to tuberculous infection.

Not only was the caseation of the hilum nodes in these rabbits very conspicuous, but the disease in the pleura was usually extensive or massive in degree. The spread of the disease by the lymph channels in all of the members of the F family is in sharp contrast to the complete absence of gross tuberculosis in the tracheobronchial nodes of the A family.

From these caseous pulmonary foci the disease spread not only by the lymph routes but even more by the blood vascular channels. Massive nodular caseous foci developed throughout both lungs (figure 3). These frequently became confluent until the lungs were consolidated by caseous pneumonia (figure 4). No encapsulation and excavation overtook these secondary lesions. Equally extensive and destructive were the large progressive caseous lesions which resulted from blood invasion of the kidneys, eyes, thyroid gland, adrenals, bone marrow, bones and joints, the peripheral lymph nodes and occasionally the brain, spleen,

ovaries and skeletal muscles (figures 3 and 4). A glance at column 10, where the number and extent of the extrapulmonary vascular metastases are listed, will show what a decisive rôle in the fate of the rabbits of this family blood invasion played.

By contrast canalicular spread was of very little import in the extension of the disease in this family. Only the intestines and trachea became involved in these rabbits as a result of passage over them of material laden with tubercle bacilli from the exudate derived from the pulmonary alveoli. Canalicular spread did not play a prominent rôle in the pathogenesis of the disease in this family because of the absence of cavities and liquefied material which could spread through preformed channels. On the other hand liquefaction and excavation did not occur in these rabbits, because of the fulminating character of the disease process.

The duration of the disease developed by these rabbits ranged from three to eight months, with an average of 4.9 months. This is only about half the length of the disease in the resistant A family.

Thus all the rabbits of this family that acquired the disease, irrespective whether they were of the second, third or fourth inbred generations, developed a rapidly progressive primary caseous pneumonic focus which soon spread to the draining tracheobronchial nodes, leading to their massive enlargement and caseation (figure 3). By contrast with the A family, where the disease remained localized to the portal of entry and slowly progressed in the lungs by canalicular spread, all the rabbits of the F family with remarkable uniformity not only disseminated their disease by the lymphatic channels but particularly by blood invasion, with resulting widespread large, nodular, destructive lesions occurring in many parts of the body (figures 3 and 4).

Microscopically the primary foci were extensive, caseous pneumonic areas in which the alveolar framework could still be discerned. Large, widely open blood vessels undergoing necrosis *en masse* were often enmeshed in these caseous zones (figure 18). The penetration of large numbers of tubercle bacilli into the blood stream by the erosion of the vessel walls was seen. Tubercle bacilli were swarming in the perifocal epithelioid cells within the alveoli (figure 15). Microscopic foci of softening, swarming with bacilli, were noted in the caseous areas, with the formation of minute sequestrating cavities (figure 13). Little or no fibrous tissue encapsulation was seen about these. The draining tracheobronchial nodes were utterly destroyed by massive caseation. Even in these secondary foci intact epithelioid cells were not prominent; most

of them succumbed to the rapidly advancing caseous process. Even the fibroblast-like cells in the abortive capsule about the nodes swarmed with tubercle bacilli. The metastatic foci in the viscera pursued the same fulminating course.

The C Family

Closely resembling the F family in its response to tuberculosis is the C family, the pedigree of which is recorded in chart 3. The tuberculosis acquired through natural respiratory contagion by members of the C family, made up wholely of either pure albino or himalaya rabbits, is recorded in table 3. A total of 17 rabbits were exposed from the second, third and fourth inbred generations, 59 per cent of these being males and 41 per cent females. Their ages at the beginning of exposure ranged from 2.5 to 13.5 months with a median age of 9.5 months.

The spread of India ink in the skin, previous to exposure, was determined in seven rabbits of the fourth inbred generation. Only one of these rabbits was a female. The spread of the dye in the skin of these rabbits ranged from 360 to 980 square mm., with a mean spread of 685. It will be remembered that the spread of India ink in these very rabbits

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The photomicrographs presented in figures 13 to 18 inclusive were made from sections stained for tubercle bacilli by the Ziehl-Neelsen method and counterstained with haematoxylin. The magnification in all of these figures is about 500.

FIG. 13. (Upper left.) The wall of a sequestrating cavity in the lung of contact C3-7 of the third inbred generation of the C family. The lumen of this cavity is shown in upper portion of the photograph. There is no fibrous tissue in the wall. It is composed of necrotic cells swarming with tubercle bacilli. The dense black spot near the edge of this cavity is a mass of bacilli. The boundary of this wall is sharp, as if cut out.

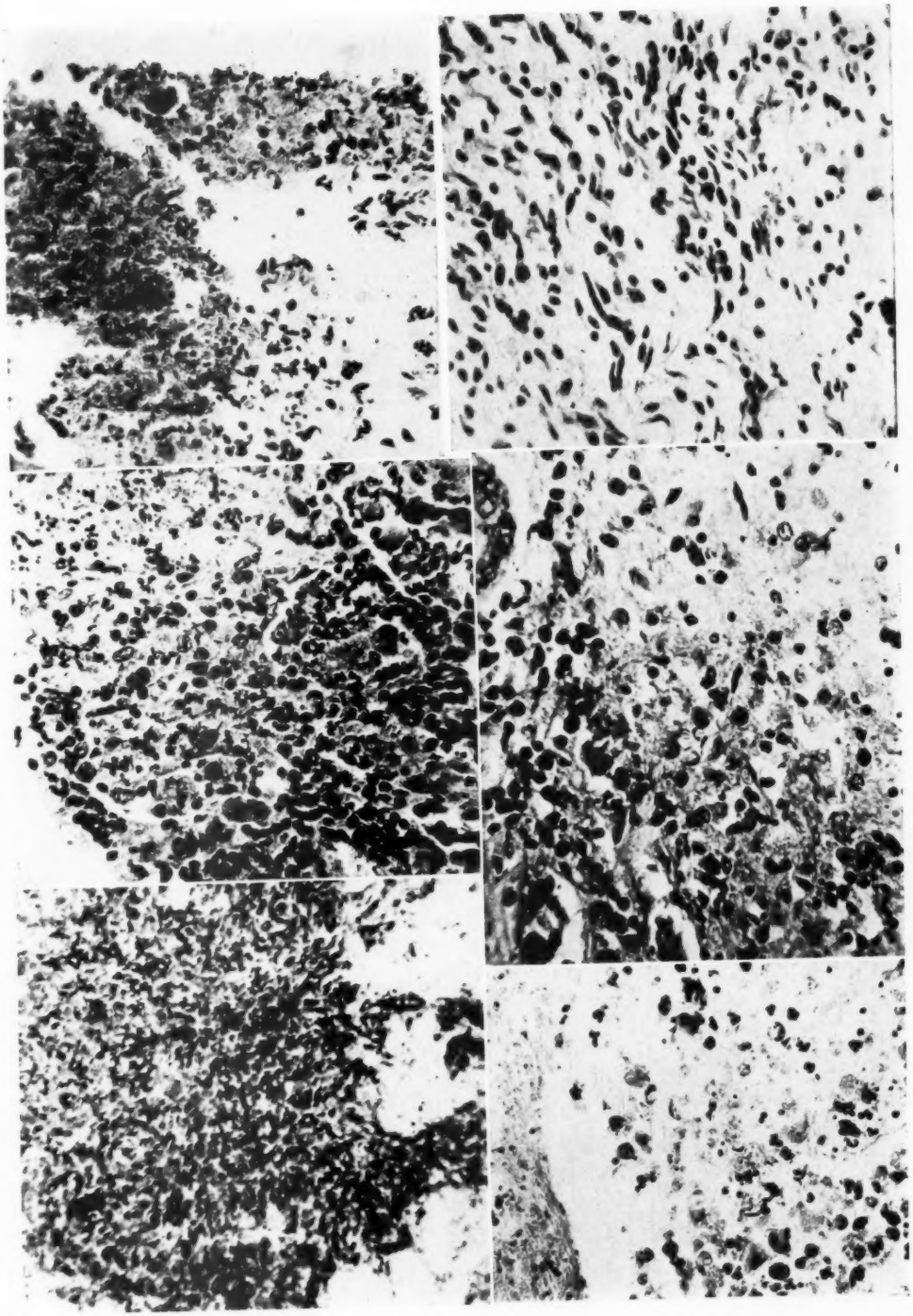
FIG. 14. (Upper right.) The wall of a cavity in the lung of contact A3-15 shown in figure 1, plate 1. It is composed of many layers of intact connective tissue cells, free from tubercle bacilli.

FIG. 15. (Center left.) The advancing edge of a caseous tubercle in the lung of contact F4-33 shown in figure 3. There are numerous tubercle bacilli in the still intact epithelioid cells lying in the alveolar spaces as well as in the interstitial tissues.

FIG. 16. (Center right.) A resolving tubercle in the lung of contact A5-3. The epithelioid cells are disintegrating, as shown near the right of the photograph. Tubercle bacilli are very rare. One microörganism is seen in the lower right corner of the photograph in close proximity to the round cells at the periphery of the healing tubercle. A zone of atelectasis surrounds this healing lesion.

FIG. 17. (Lower left.) Softening caseous tissue within a cavity of contact A3-15 shown in figure 1. The fibrous wall of this cavity is shown in figure 14. Innumerable tubercle bacilli swarm in the softening caseous tissue. In the caseous tissue not undergoing softening, as shown at the right of the photograph, bacilli are very scanty.

FIG. 18. (Lower right). The wall and lumen of an open blood vessel which has undergone caseation *en masse* in contact F4-11. The wall is completely caseous. Tubercle bacilli are seen lying free within the nonthrombosed lumen of the vessel.



FIGS. 13–18

TABLE 3

Naturally acquired respiratory tuberculous in rabbit family C

RABBIT NUMBER, FAMILY, GENERATION, SEX AND AGE AT BEGINNING OF EXPOSURE	INDIA INK SPREAD BEFORE EXPOSURE IN SQ. MM.	PRETUBERCULOUS PERIOD IN MONTHS, OR DURATION OF EXPOSURE	MAXIMUM VOLUME OF TUBERCULIN REACTION IN CMM.	CHARACTER OF PRIMARY LESION	LESIONS IN DRAINING TRACHEOBRONCHIAL LYMPH NODES	CAVITY FORMATION	CANALICULAR SPREAD	HAEMATOGENOUS AND LYMPHOGENOUS SPREAD	NUMBER AND EXTENT OF EXTRAPULMONARY VASCULAR METASTASES	DURATION OF DISEASE IN MONTHS	ANATOMICAL TYPE OF DISEASE
C2-6 ♂ 12.0 mos.	—	3.7	842	?	++	None	Slight intestinal tbc.	Scattered pulmonary tubercles; cortical tbc. of kidneys	2 moderate	3.7	Primary progressive tbc. with pleurisy and pericarditis
C2-8 ♀ 5.0	—	2.0	288	?	++	None	Ulcerative intestinal tbc. with moderate caseation of mesenteric nodes	Nodular caseous tbc. of lungs; extensive progressive renal tbc.; slight pleural tbc.	3 moderate	9.5	Primary slowly progressive generalized tbc.
C2-4 ♀ 5.0	—	7.0	0								Died; no tbc.
C2-1 ♂ 5.0	—	5.0	156	?	++++	None	Ulcerative intestinal tbc. with peritonitis and caseation of mesenteric nodes	Nodular pulmonary caseous tbc.; massive progressive renal tbc.; moderate tbc. of pleura	3 massive	3	Primary progressive generalized tbc. and caseous pneumonia
C2-7 ♂ 5.0	—	7.0	647	?	+++	None	Ulcerative intestinal tbc. with peritonitis and caseation of mesenteric nodes; tbc. of larynx	Nodular pulmonary caseous tbc.; massive progressive renal tbc.; massive pleural tbc.; tbc. of thyroid and of one eye	5 massive	5	Primary progressive generalized tbc. and caseous pneumonia
C2-15 ♂ 2.5	—	8.3	136	Caseous pneumonic focus	++++	None	Slight intestinal tbc.	Nodular fibrocaseous pulmonary tbc.; extensive progressive renal tbc.; moderate pleural tbc.; tbc. of myocardium	4 extensive	11	Primary slowly progressive tbc. with fibrocaseous pneumonia

C2-18 ♀ 2.5	—	13	240								Died; no tbc.
C3-5 ♀ 10.0	—	17	0								Killed; no tbc.
C3-4 ♀ 10.0	—	10	12	Caseous pneumonic focus	+++	None	None	Nodular pulmonary tbc.; isolated renal tubercles	2 moderate	1.5	Primary progressive tbc. with slight generalization
C3-7 ♀ 5.0	—	7	220	?	++++	None	None	Nodular pulmonary caseous tbc.; extensive pleural tbc.; progressive renal tbc.; tbc. of thyroid	4 extensive	6	Primary progressive generalized tbc. and caseous pneumonia
C4R-14 ♂ 4.0	925	4.5	10	?	++++	None	Intestinal tbc. with involvement of mesenteric nodes	Nodular pulmonary caseous tbc.; extensive pleural tbc.; progressive renal tbc.; tbc. of myocardium	4 extensive	2.7	Primary progressive generalized tbc. and caseous pneumonia
C4R-12 ♀ 9.5	980	5.5	95	?	+++	None	None	Nodular pulmonary caseous tbc.; slight pleural tbc.; progressive renal tbc.	3 extensive	1.8	Primary progressive generalized tbc. and caseous pneumonia
C4S-9 ♂ 10.0	713	3.3	200	Caseous pneumonic focus	++++	None	Isolated tubercle in appendix	Nodular caseous pulmonary tbc.; progressive renal tbc.	2 moderate	2	Primary progressive tbc. with slight generalization and empyema
C4S-11 ♂ 10.0	700	3.8	210	Caseous pneumonic focus	+	None	None	Minimal renal tbc.	2 slight	2	Primary progressive tbc. with slight generalization and snuffles
C4R-6 ♂ 10.0	576	4.0	193	?	++++	None	Ulcerative tbc. of trachea; gastrointestinal tbc.; tbc. of testis	Nodular caseous pulmonary tbc.; extensive pleural tbc.; progressive renal tbc.	3 extensive	4.5	Primary progressive generalized tbc. and caseous pneumonia

TABLE 3—*Concluded*

RABBIT NUMBER, FAMILY, GENERATION, SEX AND AGE AT BEGINNING OF EXPOSURE	INDIA INK SPREAD BEFORE EXPOSURE IN SQ. MM.	PRETUBERCULOUS PERIOD IN MONTHS, OR DURATION OF EXPOSURE	MAXIMUM VOLUME OF TUBERCULIN REACTION IN CMM.	CHARACTER OF PRIMARY LESION	LESIONS IN DRAINING TRACHEOBRONCHIAL LYMPH NODES	CAVITY FORMATION	CANALICULAR SPREAD	HAEMATOGENOUS AND LYMPHOGENOUS SPREAD	NUMBER AND EXTENT OF EXTRAPULMONARY VASCULAR METASTASES	DURATION OF DISEASE IN MONTHS	ANATOMICAL TYPE OF DISEASE
C4R-2 ♂ 13.5	540	2.7	160	Caseous pneumonic focus	++++	None	Gastro-intestinal tbc. with ulceration, peritonitis and caseation of mesenteric nodes	Nodular pulmonary caseous tbc.; progressive renal tbc.; massive pleural tbc.; tbc. of axillary nodes, pectoral and thigh muscles, subcutaneous tissues, submaxillary gland, iliac nodes, knee joint, parietal bone and cerebellum	11 massive	4.3	Primary progressive generalized tbc. and caseous pneumonia
C4R-5 ♂ 13.5	360	15	0								Killed; no tbc.

is a sex limited character (22), being greater in females than in males. Therefore, in comparing the relative spread of this dye in the various families, note must be taken of the sex of the rabbits tested. Unfortunately this sex limited character of the spread of the dye was not found until after these determinations. Hence the real significance of these figures is obscured by the sex factor, which was not adjusted in the different families. Thus in the A family 2 females and 3 males were tested, whereas in the C family this character was determined on 6 males and 1 female.

Thirteen of the 17 rabbits exposed acquired tuberculosis, an incidence of 76.5 per cent.

The pretuberculous period ranged from two to ten months. The average interval between the beginning of exposure and the acquisition of tuberculosis was 5.1 months. The maximum tuberculin sensitivity developed by these rabbits during the entire course of the acquired disease varied from very slight, with inflammation of no greater volume than 10 cmm., to severe with induration of 842 cmm. The average maximum size of the tuberculin reaction was 244 cmm., an inflammatory response which is distinctly greater than that commonly seen in the A family.

Only in 5 of the 13 rabbits that acquired tuberculosis in this family could the primary pulmonary focus be identified with some certainty. It was found especially in a disease of very short duration and consisted of a fulminating caseous pneumonic focus without encapsulation and without excavation. The draining tracheobronchial lymph nodes in these rabbits as well as in all the rabbits of this family that acquired tuberculosis were extensively to massively caseous at death. A glance at column 6 shows how uniform was the involvement of the nodes draining the portal of entry. In the rabbits that died of their tuberculosis the disease in the pleura was usually extensive. Haematogenous spread to the lungs and to the rest of the viscera played by far the most important rôle in the extension of the disease. Usually the lungs became consolidated by large, confluent, nodular, caseous pneumonic foci (figure 5). It was for this reason that the primary pulmonary focus could not be easily identified in the fatal disease. Large progressive, nodular, caseous lesions were found in the kidneys, thyroid, eyes and heart muscle. In a single instance there was in addition a destructive caseous tuberculosis of the skeletal muscles, the subcutaneous tissues, the peripheral lymph nodes, the bones and brain. Not a single gross cavity was noted in any of the 13 rabbits that acquired the disease. Canalicular spread was usually of little importance in the progress of the disease in this family.

Only in the intestines were there significant tuberculous changes that could be attributed to swallowing exudate laden with tubercle bacilli, presumably derived from the pulmonary alveoli by way of the bronchi. In only one rabbit was the larynx affected from the same source. Canalicular spread to the testis occurred only in rabbit C4-6, in the presence of progressive renal disease. A glance at column 10, where the

FIG. 5. (Upper left.) Organs of rabbit C4-2. Rapidly progressive primary generalized tuberculosis, typical of the C family. Pretuberculous period, 2.7 months. Duration of disease, 4.3 months. Confluent, nodular, caseous pneumonia of both lungs with massive caseation of both draining tracheobronchial nodes. Extensive progressive tuberculosis of both kidneys. Massive tuberculosis of diaphragmatic pleura, shown below left lung. Extensive tuberculosis of bone marrow, depicted below right lung. Caseous tubercle in skeletal muscle, seen immediately below bone marrow. Ulcerated tubercles in the appendix, in the lower left corner. Caseous tubercles in the mesenteric nodes draining the secondary intestinal tuberculosis, seen just above and to the right of the appendix. Large, caseous, nodular tubercle in the great omentum, seen to the right of the mesenteric nodes.

FIG. 6. (Lower left.) Organs of rabbit C4-4, exposed to the inhalation of about 675 "units" of tubercle bacilli in an apparatus described in the subsequent paper. Preallergic period, twenty-one days. Duration of disease, one hundred and sixty days. Primary, slowly progressive generalized tuberculosis with nodular caseous pneumonia and calcification, a disease of greater chronicity than is characteristic of the majority of members of this family. Extensive, yet discrete caseous tuberculosis of both tracheobronchial nodes. Chronic medullary tuberculosis of both kidneys. Tuberculous ulceration of the trachea. Tuberculosis of the cortex of the brain. Solitary tubercle in thyroid gland below right lung. Minute gray tubercle in adrenal, depicted immediately below thyroid gland. Tuberculosis of cornea and lachyrmal gland. Tuberculosis of marrow of ribs. Solitary tubercle in ovary, shown to the right of section of costal pleura. Extensive tuberculosis of appendix, seen in the right lower corner. Isolated tubercle in colon, seen in the left lower corner.

FIG. 7. (Lower right.) Organs of rabbit H2-2 of the second inbred generation of the H family. Pretuberculous period, six months. Duration of disease, three months. Primary rapidly progressive, generalized tuberculosis with miliary involvement of the lungs. Large primary, caseous focus with beginning softening in the upper lobe of the right lung, accompanied by extensive enlargement and massive caseation of the draining tracheobronchial nodes. Progressive renal tuberculosis. Uniformly distributed, bilateral, haematogenous, miliary pulmonary tuberculosis. Extensive tuberculosis of diphragmatic pleura, shown in the left lower corner. To the right of this structure may be seen discrete caseous tuberculosis of mesenteric nodes, draining secondary intestinal tuberculosis not shown in figure. Tuberculosis of the bone marrow, of the femur and of the ribs, shown in lower right corner.

FIG. 8. (Upper right.) Organs of rabbit H4-1, of the fourth inbred generation of the H family. Slowly progressive generalized tuberculosis of intermediate type. Pretuberculous period, three months. Duration of disease, nine months. Anterior surfaces of the lungs face the reader. Small encapsulated cavity in the middle of the left lung, on the right side of the photograph. A minute, caseous focus can be seen in the small, homolateral, draining tracheobronchial node. Slight, nonprogressive, pulmonary, nodular tuberculosis of haematogenous origin, uniformly distributed in both lungs. Massive tuberculosis of diaphragmatic pleura, shown below lung on left side of photograph. Extensive tuberculosis of appendix and nodular tuberculous peritonitis in the omentum, seen in lower right corner. Minute cortical tubercles in kidneys. Tuberculosis of epididymis of tubular origin.

FIGS. 5-8

number and extent of the extrapulmonary vascular metastases are listed, shows that usually few internal organs were invaded, but those involved were extensively to massively affected. In all but 3 of the rabbits that died of their acquired tuberculosis the disease lasted for two to five months. The average duration of the disease in all the rabbits in the C family that died of their tuberculosis, was 5.3 months. This is an illness of essentially the same duration as seen in the F family.

It is plain, therefore, that the pathogenesis of the disease in the C family closely resembles that of the susceptible F family and, like it, differs from that of the resistant A family in every respect. However, the progression and duration of the disease were less uniform in the C than in the F family. Occasional rabbits of the C family were encountered in which the disease, while progressing in the essential manner of a first infection, yet was remarkably slow (figure 6). This is illustrated in rabbits C2-15 and C2-8, in which the illness lasted eleven and 9.5 months respectively.

Similarly, microscopic analysis showed that in the majority of rabbits of this family there was the same destructive caseous pneumonic process with sequestrating cavities, the ragged edges of which contained numerous bacilli (figure 13), the same invasion of blood vessels and the same alveolar plugs swarming with tubercle bacilli as found in the F family. However, in some rabbits of this family there were large stretches of intact epithelioid cells with few bacilli, obliterating endarteritis of vessels, some fibrosis and occasional foci of calcification. Yet even in these relatively resistant individuals no typical cavities with well formed fibrous walls were seen.

The H Family

Of particular interest, from the standpoint of the rôle of the genetic constitution in tuberculosis, is the H family. A glance at the eleventh column in table 4 will show that the rabbits of this family naturally fell into two groups. Those of the second inbred generation acquired a rapidly fatal disease lasting from three to four months. Those of the third and fourth inbred generations on the other hand, acquired a disease which usually killed them in six to nine months.

Yet all these rabbits were descendants of the same original parents H1-1 and H1-2. However, as may be seen in chart 8, and as analyzed at the beginning of this paper, there is reason to believe that in the constitution of the second inbred generation of the H family the characters

of rabbits 1 and 27, the preponderant ancestors of the susceptible families F and C, may have played a dominant rôle. In the third generation of this family however, the characteristics of the remaining, possibly more resistant ancestors of the H family may have come to the fore; for they greatly outnumbered the susceptible ancestors 1 and 27. It must be emphasized at this point that as yet there is no available outward sign of resistance or susceptibility. It is possible, therefore, that in breeding the third and fourth inbred generations, the more resistant stock was blindly chosen for the propagation of this family.

For these reasons it is advisable to consider the second inbred generation of this family in one group and the third and fourth generations in another.

The second inbred generation of the H family: Eight rabbits of the second inbred generation, all descendants of rabbits H1-1 and H1-2, were exposed simultaneously with the corresponding inbred generation of all the other families. Of these, 2 were females and 6 were males. Their ages ranged from 2.5 to six months with a median of 5. The interval elapsing between the beginning of the exposure and the onset of contact tuberculosis varied between four and 8.3 months with an average of six months.

Of the 8 rabbits of the second inbred generation of the H family that were exposed for a period of five or more months, 6, or 75 per cent, acquired tuberculosis. The number of rabbits is admittedly small, but, as will be seen, the uniformity of their response makes the observations significant.

The maximum size of the tuberculin reaction developed by these rabbits during the entire duration of their acquired tuberculosis varied from 120 to 691 cmm. of reddened induration, with an average of 316 cmm. It will be noted that this sensitivity is practically of the same average intensity as that of the uniformly highly susceptible F family and is much above that developed by the resistant A family.

The primary focus developed by these rabbits was uniformly a single, somewhat encapsulated, caseous pneumonic area with central softening but without actual excavation. The tracheobronchial lymph nodes draining this portal of entry were usually extensively to massively enlarged and caseous (figure 7).

From these primary pulmonary foci the disease extended to the remainder of the lungs by the haematogenous route in the form of discrete or conglomerate nodular, caseous miliary tubercles (figure 7). The

pleura became extensively involved in all instances. Extensive and progressive renal tuberculosis was present in all contacts that died from the disease. Especially destructive were the tuberculous lesions of haematogenous origin in the eyes, lymph nodes and particularly the bone marrow. A glance at column 10, where the number and extent of the extrapulmonary vascular metastases are recorded, shows that the degree of haematogenous spread was almost as extensive as in the C family.

Canalicular spread played but a minor rôle in the fate of these rabbits. Usually it was present in the form of inconspicuous lesions in the intestines. It is noteworthy that even these secondary intestinal lesions gave rise to caseation of the mesenteric nodes, indicating the low degree of resistance of this generation of the H family. In a single instance canalicular spread accounted for tuberculosis of the larynx and testis.

All these rabbits died uniformly in a very short time ranging from three to 4.5 months with an average duration of illness of 3.6 months. It is the relatively shorter duration of the disease in the second generation of the H family that is obviously responsible for whatever differences were observed between these rabbits and those of families F and C. It shared with these all the essentials of the pathogenesis of the disease. Like the F's and C's, 100 per cent of the second generation of the H family developed the progressive first infection type of tuberculosis, with rapid spread of the disease by lymphogenous and haematogenous routes from a primary pulmonary focus. However death overtook these rabbits more quickly than those of the F and C families. This explains the miliary character of the pulmonary disease in this generation of the H family (figure 7), and the caseous pneumonia which characterized the pulmonary tuberculosis in the former families. Despite all these similarities, however, it is noteworthy that encapsulation and softening of the primary focus occurred more frequently in this group of rabbits than in the F and C families. Further light on the pathogenesis of the disease in this generation of the H family was given by the microscopic study of these animals, considered below.

The third and fourth inbred generations of the H family: Eight rabbits of the third and fourth inbred generations of the H family were exposed for a period of five to seventeen months. Three of these were females and 5 were males. Their age varied between seven and twelve months with a median of 8.5.

Of these 8 rabbits, 5 or 62.5 per cent acquired tuberculosis.

A comparison of the maximum tuberculin sensitivity developed by the rabbits of the second generation of the H family that acquired tuberculosis, on the one hand, and those of the third and fourth generations, on the other hand, is interesting. Unfortunately too few observations are available to make them statistically significant. Nevertheless it is suggestive that the second generation H rabbits developed a considerably greater tuberculin sensitivity than those of the third and fourth generations of this family. Reactions in the former averaged 332 cmm. in area, with a range of 120 to 691; in the latter they averaged 187 cmm. with a range of 107 to 276. Thus the intensity of allergy during the course of naturally acquired tuberculosis again tended to be greater in the more susceptible generation. It will be recalled that the highly susceptible F and C families tended to develop a decidedly higher degree of tuberculin sensitivity than the highly resistant A family. Furthermore this tuberculin sensitivity tended to be much greater in the second generation H family than that in the A family, whereas there was practically no difference between the A family and the third and fourth generations of the H family in this respect.

The primary focus of these rabbits consisted of thin walled cavities in a number of instances, suggesting a greater chronicity of the disease at the portal of entry of the bacilli in these generations as compared with the lesions in members of the second generation of this family (figures 7 and 8). Correspondingly, the tracheobronchial lymph nodes were usually little affected (figure 8). In H3-5 these nodes were free from gross tuberculosis altogether. In three only isolated caseous foci were found, and in one only was there a moderate tuberculosis. Thus lymphogenous dissemination of the disease was strikingly less pronounced in these generations than in the second generation of this family.

Haematogenous spread played a prominent rôle in the extension of the disease in these rabbits. However, despite the long duration of the disease in the majority of these rabbits, the haematogenous pulmonary tubercles remained discrete (figure 8). The pleural disease was usually quite extensive. The tuberculosis disseminated by the blood stream to other organs was not uniform in its progress. In some it caused extensive rapidly progressive renal disease. In others, the kidneys were the seat of a chronic ulcerative tuberculosis. Besides, there were metastases to the sclerae, bones, brain and lymph nodes of these rabbits; even the skeletal muscles were invaded in one rabbit.

Canalicular spread also played a considerable rôle in the extension of

the disease in some of these rabbits. Extensive intestinal tuberculosis was found in most of them. In some the extension of the intestinal disease to the peritoneum gave rise to tuberculous peritonitis (figure 8). The larynx and epiglottis were affected in half of these rabbits, presumably from the same origin as the intestinal disease. Similar canalicular spread from the renal tuberculosis extended the disease to the testis in two (figure 8).

A glance at column 10, in table 4, where the number and extent of the extrapulmonary vascular metastases are listed, shows that an average of 4 organ systems were affected by this process and that the degree of involvement was from moderate to extensive. This is distinctly less than was found in the rabbits of the second generation of this family.

The duration of the disease in 4 of these rabbits varied from six to nine months; in one, it lasted only 3.8 months. The average illness of all of these rabbits of the third and fourth generations was 6.5 months as contrasted with an average duration of 3.6 months in the second generation of the H family. Two of these rabbits developed the primary progressive type of tuberculosis. One rabbit died of tuberculosis anatomically of the reinfection type and two succumbed to an infection intermediate in anatomical type between primary and reinfection type tuberculosis.

Thus the second generation of the H family was uniform in its response to tuberculosis, invariably susceptible and developed a primary progressive generalized tuberculosis similar in its essential characters to that developed by the susceptible families F and C (figure 7). The third and fourth generations of the H family were generally definitely more resistant but with considerable variation from individual to individual. Most of them exhibited some chronicity with a tendency for excavation of the primary focus with relatively effective localization of the disease at the portal of entry, the draining nodes being but slightly or not at all affected, with moderate haematogenous dissemination and prominent canalicular spread, a disease characteristic of the reinfection or intermediate type (figure 8). In the remaining rabbits, however, the disease was essentially of the same nature as the second generation of the H family, i.e. primary progressive generalized tuberculosis.

Microscopic analysis of tissues of members of the H family showed that the fundamental difference in the response of the second generation on the one hand, and the third and fourth generations on the other, was the relative chronicity of the primary focus. In the second generation this

TABLE 4

Naturally acquired respiratory tuberculosis in rabbit family H

RABBIT NUMBER, FAMILY, GENERATION, SEX AND AGE AT BEGINNING OF EXPOSURE	INDIA INK SPREAD BEFORE EXPOSURE IN SQ. MM.	PRETUBERCULOUS PERIOD IN MONTHS, OR DURATION OF EXPOSURE	MAXIMUM VOLUME OF TUBERCULIN REACTION IN CMM.	CHARACTER OF PRIMARY LESION	LESIONS IN DRAINING TRACHEOBRONCHIAL LYMPH NODES	CAVITY FORMATION	CANALICULAR SPREAD	HAEMATOGENOUS AND LYMPHOGENOUS SPREAD	NUMBER AND EXTENT OF EXTRAPULMONARY VASCULAR METASTASES	DURATION OF DISEASE IN MONTHS	ANATOMICAL TYPE OF DISEASE
H2-5 ♀ 4.0 mos.	—	5									Died; no tbc.
H2-7 ♂ 6.0	—	4.5	375	Caseous pneumonic focus with softening	+++	None (softening)	Slight tbc. of intestines with caseation of mesenteric nodes; tbc. of larynx and testis	Conglomerate miliary pulmonary tubercles; extensive pleural tbc.; progressive renal tbc.; tbc. of left eye and bone marrow	5 extensive	3	Primary progressive generalized tbc.
H2-8 ♀ 6.0	—	5									Killed; no tbc.
H2-1 ♂ 6.0	—	5	176	Single caseous pneumonic focus with softening	+++	One	Isolated caseous focus in appendix	Scattered nodular caseous pulmonary tbc.; extensive pleural tbc.; progressive renal tbc.; caseous tbc. of bone marrow	4 extensive	3.6	Primary progressive generalized tbc.
H2-2 ♂ 6.0	—	6.0	150	Single caseous pneumonic focus with softening	++++	None (softening)	Moderate intestinal tbc. with caseation of mesenteric nodes	Miliary pulmonary tbc.; moderate pleural tbc.; progressive renal tbc.; tbc. of bone marrow and lymph nodes in general	5 extensive	3.0	Primary progressive generalized tbc.

TABLE 4—*Concluded*

RABBIT NUMBER, FAMILY, GENERATION, SEX AND AGE AT BEGINNING OF EXPOSURE	INDIA INK SPREAD BEFORE EXPOSURE IN SQ. MM.	PRETUBERCULOUS PERIOD IN MONTHS, OR DURATION OF EXPOSURE	MAXIMUM VOLUME OF TUBERCULIN REACTION IN CMM.	CHARACTER OF PRIMARY LESION	LESIONS IN DRAINING TRACHEOBRONCHIAL LYMPH NODES	CAVITY FORMATION	CANALICULAR SPREAD	HAEMATOGENOUS AND LYMPHOGENOUS SPREAD	NUMBER AND EXTENT OF EXTRAPULMONARY VASCULAR METASTASES	DURATION OF DISEASE IN MONTHS	ANATOMICAL TYPE OF DISEASE
H2-17 ♂ 2.5	—	8.3	120	Caseous pneumonic focus	++++	None	Slight tbc. of appendix with caseation of mesenteric nodes and extensive peritonitis	Nodular pulmonary caseous tbc.; extensive pleural tbc.; progressive renal tbc.; caseous, softening tbc. of axillary nodes; tbc. of iris and bone marrow	6 extensive	4.5	Primary progressive generalized tbc. and caseous pneumonia
H2-18 ♂ 2.5	—	4.0	384	?	++++	None	Slight intestinal tbc. with discrete caseation in mesenteric nodes	Miliary pulmonary tbc.; extensive pleural tbc.; extensive progressive renal tbc.; tbc. of lymphatic system	4 extensive	4.5	Primary progressive generalized tbc.
H2-20 ♂ 2.5	—	8	691	?	++	None	None	Miliary pulmonary tbc.; extensive pleural tbc.; extensive progressive renal tbc.; miliary tbc. of bone marrow	4 extensive	3.0	Primary progressive generalized tbc.
H3-3 ♀ 8.5	—	17									Killed; no tbc.
H3-2 ♂ 8.5	—	16								.	Died; no tbc.
H3-4 ♂ 7.0	—	11									Died; no tbc.

H3-5 ♀ 7.0	—	5	129	?	0	None	Gastro-intestinal tbc. with ulceration and caseation of peri-portal nodes	Nodular fibrocaseous pulmonary tbc.; extensive pleural tbc.; progressive renal tbc.	2 extensive	6	Slowly progressive generalized tbc. of "re-infection" type
H4-13 ♀ 7.0	825	3.6	107	Single caseous pneumonic focus with softening	++	None	Extensive intestinal tbc.; extensive tbc. of larynx and epiglottis	Miliary pulmonary tbc.; extensive pleural tbc.; extensive progressive renal tbc.	3 extensive	3.8	Primary progressive generalized tbc.
H4-4 ♂ 12.0	770	3.6	158	Caseous pneumonic focus	+	None	Intestinal tbc. with ulceration	Nodular pulmonary caseous tbc.; moderate pleural tbc.; few cortical tubercles in kidneys; tbc. of sclera of both eyes, popliteal nodes, parietal bone and tuberculoma of brain	7 moderate	6.5	Primary slowly progressive generalized tbc.
H4-3 ♂ 12.0	598	2.6	276	One thin walled cavity	+	Numerous, small	Intestinal tbc. with peritonitis; extensive ulcerative fibrocaseous tbc. of larynx and epiglottis; tbc. of ureter and testis	Nodular pulmonary tubercles, some excavated; moderate pleural tbc.; chronic ulcerative renal tbc.; tbc. of muscle of chest	4 moderate	7.0	Slowly progressive generalized tbc. of intermediate type
H4-1 ♂ 12.0	396	3.0	264	Two thin walled cavities	+	Two	Extensive intestinal tbc. with peritonitis; extensive tbc. of larynx and epiglottis; extensive tbc. of one testis	Discrete pulmonary nodular tubercles; massive pleural tbc.; chronic fibrous renal tbc.; tbc. of popliteal nodes	4 extensive	9.0	Slowly progressive generalized tbc. of intermediate type

focus was encapsulated but the connective tissue wall underwent progressive caseation. In the third and fourth generations the connective tissue wall was more enduring. Within this capsule the primary focus underwent softening, liquefaction or calcification. Outside of this and its consequences, there was little difference in the histogenesis of the disease in these generations. In both intact epithelioid cell areas with few bacilli and only sparse caseation were common. Notable interfocal lymphocytic and fibrotic deposits were often seen. Multiplication of bacilli occurred in minute foci of softening as in the A family. This indicates considerable efficacy on the part of the epithelioid cells of this family in suppressing the growth of the bacilli. The vulnerable system of the H family was its blood vessels. There was little tendency to deposit connective tissue about their walls in contiguity with the tuberculous process. Frequently these foci involved the wall with the formation of epithelioid cells in the media. These gradually pushed and stretched the overlying endothelial lining until it desquamated and the tuberculous tissue, which caseated at times, was swept into the blood stream. This process tended continuously to carry the bacilli to different parts of the body. Bronchogenic spread was present but not prominent. In the second generation the tracheobronchial nodes were frequently extensively caseous with numerous bacilli in the peripheral epithelioid cells. In the third and fourth generations, despite extensive pulmonary involvement in some cases, there was little or no tuberculosis in these nodes.

The D Family

By contrast with the H family, where the third and fourth inbred generations responded to tuberculous infection in a manner quite different from that of the second generation, the D family consisted of rabbits of more uniform response to tuberculosis. The pedigree of the D family is given in chart 4 and their reaction to contact tuberculosis is recorded in table 5.

A total of 12 rabbits, males and females in equal number, were exposed. They were derived from the second, third, fourth and fifth inbred generations with an age range of 2.5 to twelve months and a median of eight. Four rabbits, 2 males and 2 females of the fifth inbred generation, were tested with India ink. The spread of the dye in the skin of these rabbits ranged from 800 to 1120 square mm. with an average of 921. The

average spread of the dye in 5 other rabbits of this family was 935. The detailed observations on these rabbits are given in paper III of this series. This extent of permeation of the skin is among the greatest seen in any of the families. If the spread of tuberculosis were related to this single factor one might expect that the D family should be among the most susceptible. However, as was already clearly seen above, the C and H rabbits of the fourth inbred generations do not differ in their relative capacity to spread the dye in their skin and yet differ greatly in their resistance to tuberculosis. As will become plain in the course of this report, no single factor thus far analyzed alone determines resistance. Resistance or susceptibility to tuberculosis is evidently controlled by a group of determinants.

Of the 12 rabbits exposed for a period of eight to nineteen months, 7 or 58.3 per cent acquired tuberculosis.

The pretuberculous period varied from 3.8 to eight months with an average interval of 5.9 months elapsing between the beginning of exposure and the onset of the disease.

The primary focus could be identified with some certainty in only 3 of these rabbits. In 2 rabbits caseous pneumonic areas were found, in one case with and in the other without softening. In the third the primary focus was an encapsulated pulmonary cavity. There were 2 other rabbits in whose lungs there were numerous cavities one or more of which might have been the primary lesions at the portal of entry. Thus the rabbits of the D family responded at the portal of entry with either a rapidly progressive caseous pneumonic focus or a chronic ulcerative lesion. Correspondingly the lymph nodes draining the portal of entry were either extensively caseous or but little involved. A glance at the fifth, sixth and seventh columns of table 5 shows that usually the disease in the tracheobronchial nodes was a reflection of the chronicity of the disease in the primary focus. With acutely progressive primary pulmonary lesions the hilum nodes were extensively affected; with a slowly progressive, ulcerative, primary pulmonary lesion the disease in the draining nodes was absent or slight.

In 6 of the 7 rabbits that acquired tuberculosis the dissemination of the disease to the remainder of the lung took place by the haematogenous route with the development of confluent nodular caseous pneumonia in 2, and discrete miliary or larger nodular subpleural lesions in the remaining 4 animals. In one rabbit the disease extended in the lungs by canilicular spread as well, as suggested by the numerous pulmonary

TABLE 5

Naturally acquired respiratory tuberculosis in rabbit family D

RABBIT NUMBER, FAMILY, GENERATION, SEX AND AGE AT BEGINNING OF EXPOSURE	INDIA INK SPREAD BEFORE EXPOSURE IN SQ. MM.	PRETUBERCULOUS PERIOD IN MONTHS, OR DURATION OF EXPOSURE	MAXIMUM VOLUME OF TUBERCULIN REACTION IN CMM.	CHARACTER OF PRIMARY LESION	LESIONS IN DRAINING TRACHEOBRONICAL LYMPH NODES	CAVITY FORMATION	CANALICULAR SPREAD	HAEMATOGENOUS AND LYMPHOGENOUS SPREAD	NUMBER AND EXTENT OF EXTRAPULMONARY VASCULAR METASTASES	DURATION OF DISEASE IN MONTHS	ANATOMICAL TYPE OF DISEASE
D2-2 ♂ 4.0 mos.	—	5	405	Caseous pneumonic softened foci	++++	Two	Gastro-intestinal tbc. with ulceration and caseation of mesenteric nodes	Nodular pulmonary caseous tbc.; massive renal tbc.; extensive pleural tbc.; caseous tbc. of scapula and miliary tubercles in liver	5 massive	5.8	Primary progressive generalized tbc. and caseous pneumonia
D2-5 ♀ 4.0	—	8	90	?	0	Numerous	Numerous pulmonary cavities; tbc. of larynx and intestines	Non progressive renal tbc.; extensive tbc. of pleura and eye	3 moderate	5.7	Ulcerative pulmonary phthisis of "reinfection" type
D2-4 ♀ 4.0	—	8	102	?	+	Numerous	Numerous pulmonary cavities; tbc. of larynx and of intestines with ulceration in the latter	Miliary-pulmonary tubercles; extensive chronic renal tbc.; slight pleural tbc.; uterine tbc.	4 moderate	5.6	Ulcerative pulmonary phthisis of intermediate type
D3-6 ♀ 2.5	—	19	0								Killed; no tbc.
D3-7 ♂ 2.5	—	19	0								Died; no tbc.
D4-4 ♂ 12.0	—	17									Killed; no tbc.

D4-3 ♂ 12.0	—	17									Killed; no tbc.
D4-6 ♀ 12.0	—	4	8	?	++++	None	Slight tbc. of trachea and appendix	Nodular pulmonary caseous tbc.; slight nonprogressive renal tbc.; slight pleural tbc.; tbc. of ovary and conjunctiva	5 moderate	6.7	Primary progressive tbc. and caseous pneumonia
D6-S1 ♀ 9.7	1120	3.8	583	Pulmonary cavity	++	Two	Slight intestinal tbc.	Discrete, subpleural, stellate tubercles in lung; slight chronic renal tbc.; slight pleural tbc.; extensive tbc. of mammae, axillary nodes and bone marrow	6 moderate	4.6	Slowly progressive tbc. of intermediate type and pneumonia
D6-S5 ♂ 6.0	806	6.5	372	Caseous pneumonic focus	+++	None	Ulceration of trachea; tbc. of intestines	Nodular pulmonary tuberculosis; slight chronic renal tbc.; tbc. of axillary and popliteal nodes; tbc. of scapula and joints	6 moderate	8	Slowly progressive generalized primary tbc.
D6-R3 ♂ 12.0	800	5.7	276	?	+	None	Minimal appendicular tbc.	Nodular pulmonary tubercles; slight renal tbc.; minimal pleural tbc.; tbc. of popliteal node	4 slight	6	Slowly progressive generalized tbc. of intermediate type plus snuffles
D6-R5 ♀ 10.0	960	15	+								Killed; no tbc.

cavities. In another contact canalicular spread from excavated pulmonary foci was the only moder of dissemination to the remainder of the lung. The disease in the pleura was extensive in 3 and slight or negligible in 4 rabbits.

From the pulmonary foci haematogenous spread occurred in the kidneys in all instances. This resulted in progressive disease in 2 rabbits and in slight chronic tuberculosis in 5. In some rabbits, besides the kidneys, the bone marrow, the bones and joints, the eyes, the liver, the mammary gland, the uterus, the ovary, and the popliteal and axillary nodes were the seat of tuberculosis, which was usually non-progressive. Column 10 of table 5 lists the number and extent of the extrapulmonary vascular metastases. As will be observed, from 3 to 6 organ systems were invaded, but usually the tuberculosis in these organs was only moderate in extent.

Some canalicular extension occurred in every case, but in only 3 instances did this mode of spread play a significant rôle in the fatal outcome of the disease. The canalicular extension in the lungs has already been mentioned. In addition, the larynx, trachea and intestines were involved, with ulceration in the latter two.

Primary progressive generalized tuberculosis killed 3 rabbits in 5.8, 6.7 and eight months respectively. One rabbit died of ulcerative pulmonary phthisis, of the reinfection type, in 5.6 months. In the remaining 3 rabbits the tuberculosis was of a type intermediate between these two. Two of these died of pneumonia complicating the tuberculosis. The average duration of illness in all these rabbits was 6.1 months.

It is seen that the D family, like the third and fourth generations of the H family, is intermediate in resistance to tuberculosis between the highly resistant family A, on the one hand, and the highly susceptible F and C families and the second generation of the H family, on the other hand. Again we see that the members of the D family are not homogeneous, some responding to tuberculosis like the A rabbits (figure 10) and others like the F rabbits (figure 9). But the resistance of the more resistant D's falls short of the resistance of the A's, and the susceptibility of the susceptible members of the D family is not as pronounced as that of the F and C families.

Microscopic examination of the D family confirmed the gross observations. In some rabbits tubercle bacilli swarmed in the epithelioid cells on the periphery of the tuberculous nodules. However no sequestration cavities developed; on the contrary, thick walled, encapsulated, pus-containing cavities formed. Slow seepage of tubercle bacilli into the

blood occurred as a result of an endarteritis as described in the H family. Sudden flooding of the circulation by the rupture of tubercles projecting into the lumen of vessels was also encountered. The tubercles of haematogenous origin in distant organs such as the bones and joints at times underwent softening with bacilli swarming in the pus. The tracheobronchial lymph nodes were extensively caseous in some rabbits with bacilli in great numbers apparently multiplying within the living epithelioid cells. In other rabbits bacilli were few; and poorly stained shadows were seen in the softening pulmonary foci. Liquefaction and calcification at times overtook these areas of softening. Bacilli have been found even in the calcified areas. The walls of these cavities were thick and fibrous. Much fibrous tissue was deposited about the vessels and in concentric circles within their interior in areas of contiguity with the softening tissue. The vessels enmeshed in caseous tissue were thrombosed; no open vessels were met. The metastatic tubercles in the various organs were chiefly "hard" tubercles with mature epithelioid cells, little caseation and few visible bacilli. Canalicular spread played a conspicuous rôle in the extension of the disease in the lungs and the urogenital system of these rabbits.

This brings us to the last family which we shall consider in this presentation, family B. For, while some data at hand suggest that the E and G families belong to the group of intermediate resistance, not enough evidence is now available to place them categorically in one or another division.

The B Family

In table 6 are recorded the rabbits of the B family from the second, third, fourth and fifth inbred generations. The pedigree of these rabbits is given in chart 2. These were exposed simultaneously with members of inbred generations of the remaining families. Altogether 14 rabbits of the family were exposed. Five of these were males and 9 were females. The age of these rabbits at the beginning of exposure varied from 2.5 to twelve months, with a median age of 4.7. Four rabbits of the fifth inbred generation were injected intracutaneously with India ink. The spread of the dye in their skin varied from 322 to 1290 square mm., the mean being 897 as compared with the mean of 546 for the A family. The difference has a P value of 93 which is of some statistical significance.

Ten of the 14 rabbits exposed acquired tuberculosis, an incidence of 71 per cent.

The average interval elapsing between the beginning of the exposure

TABLE 6

Naturally acquired respiratory tuberculosis in rabbit family B

RABBIT NUMBER, FAMILY, GENERATION, SEX AND AGE AT THE BEGINNING OF EXPOSURE	INDIA INK SPREAD BEFORE EXPOSURE IN SQ. MM.	PRETUBERCULOUS PERIOD IN MONTHS, OR DURATION OF EXPOSURE	MAXIMUM VOLUME OF TUBERCULIN REACTION IN CMM.	CHARACTER OF PRIMARY LESION	LESIONS IN DRAINING TRACHEOBRONICAL LYMPH NODES	CAVITY FORMATION	CANALICULAR SPREAD	HAEMATOGENOUS AND LYMPHOGENOUS SPREAD	NUMBER AND EXTENT OF EXTRAPULMONARY VASCULAR METASTASES	DURATION OF DISEASE IN MONTHS	ANATOMICAL TYPE OF DISEASE
B2-6 ♀ 4 mos.	—	6.0	0	Caseous pneumonic focus	++	None	None		1 moderate	2.0	Primary complex
B2-4 ♀ 4.0	—	5.0	184	Caseous pneumonic focus	+++	None	None	Moderate pleural tbc.; isolated caseous cortical tbc. of kidneys; nodular pulmonary caseous tbc.,	3 moderate	3	Primary progressive tbc. and pneumonia
B2-3 ♀ 4.0	—	13	24	One firmly walled off purulent cavity	+++	Two	Tbc. of lungs, intestines and mesenteric nodes	Extensive cortical tbc. of kidneys; discrete, nodular, caseous pulmonary tbc.	2 extensive	4	Primary progressive tbc., caseous pneumonia and cavity
B2-5 ♀ 4.0	—	8	153	?	0	Moderate number	Tbc. of pharynx and intestines with caseation of mesenteric nodes	Discrete caseous and excavated pulmonary tubercles; fibrous cortico-medullary renal tbc.; extensive pleural tbc.; tbc. of both eyes	3 moderate	11.5	Chronic tbc. of "reinfection" type
B3-7 ♀ 2.5	—	19	0								Died; no tbc.
B3-8 ♀ 2.5	—	4.6	176	One encapsulated purulent cavity	+	One	Tbc. of intestines with peritonitis; tbc. of larynx	Moderate pleural tbc.; tbc. of mammae, knee joint, bone marrow and slight cortico-medullary renal tbc.; miliary pulmonary tbc.	6 moderate	7	Slowly progressive tbc. of intermediate type

				cavity				medullary renal tbc.; miliary pulmonary tbc.			
B4-11 ♀ 9.5	—	17	0								Killed; no tbc.
B4-9 ♂ 9.5	—	17	0								Killed; no tbc.
B4-15 ♀ 9.5	—	7	58	Two caseous pneumonic foci	+	None		Miliary pulmonary tubercles	1 slight	1.3	Primary complex of intermediate type and pneumonia
B4-7 ♂ 9.5	—	3	96	Two encapsulated purulent cavities	++	Two	Tbc. of lungs, stomach and testis	Subpleural pulmonary tubercles; slight pleural tbc.; tbc. of bones and joints, and chronic renal tbc.	5 moderate	7	Slowly progressive tbc. of intermediate type
B5-1 ♂ 12.0	925	8.5	80	Caseous pneumonic focus with softening	+++	None	Tbc. of gastro-intestinal tract	Nodular, pulmonary, caseous tbc.; massive pleural tbc.; tbc. of axillary nodes and lymph nodes generally; extensive progressive renal tbc.; submiliary tubercles in liver	6 extensive	4	Primary progressive tbc. and caseous pneumonia
B5-4 ♂ 12.0	322	15	0								
B5-13 ♀ 5.5	1290	3.8	86	Caseous pneumonic focus	++++	None	Ulcerative tbc. of larynx, epiglottis, trachea and intestines	Nodular caseous tbc. in lungs; moderate chronic cortico-medullary tbc. in kidneys; slight pleural tbc.; tbc. of muscles and adrenal; tuberculoma of brain; meningeal tbc.	7 moderate	6.2	Primary progressive generalized tbc.
B5-20 ♂ 3.0	1050	6.0	348	Caseous pneumonic focus with softening	++++	None	Ulcerative intestinal tbc.	Discrete pulmonary tubercles; slight renal fibrosing tbc.; tbc. of bone marrow, of maxilla and of mesentery of testis; moderate tbc. of pleura	6 moderate	7	Primary progressive generalized tbc.

FIGS. 9–12

and the onset of the acquired tuberculosis was 6.5 months, with a range of 3.8 to thirteen months. No significant difference was observed in the pretuberculous period in the A, B and other families.

In table 6, column 4 it may be seen that the maximum tuberculin sensitivity developed by the B rabbits which acquired tuberculosis was low. In half of these the maximum volume of the inflammation resulting from tuberculin injection was less than 100 cmm. during the entire course of the disease. In 3 it was less than 200. Only rabbit B5-20 showed a high degree of tuberculin sensitivity, whereas rabbit B2-6 showed no positive tuberculin reaction, although he had a primary complex with

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FIG. 9. (Upper left.) Organs of rabbit D2-2. Primary progressive generalized tuberculosis. Pretuberculous period, five months. Duration of disease, 5.8 months. Nodular caseous pulmonary tuberculosis with zones of confluent caseous pneumonia. Massive enlargement and caseation of both tracheobronchial nodes. In lower right corner is depicted ulcerative tuberculosis of the colon; above it and to the left are seen discrete caseous tubercles in the mesenteric nodes draining the secondary intestinal tuberculosis. Massive voluminous caseous lesions in the cortex of both kidneys with cavity in left. Extensive tuberculosis of diaphragmatic pleura, shown in the lower left corner.

FIG. 10. (Upper right.) Organs of rabbit D6-S1, labeled in the photograph as Di5-S1. Slowly progressive tuberculosis of intermediate type. Pretuberculous period 3.8 months. Death from intercurrent pneumonia in 4.6 months. Thick walled cavity in the lower lobe of the right lung posteriorly with small, discrete, caseous foci in the tracheobronchial nodes. Scattered, stellate, nonprogressive tubercles in both lungs. Minimal, miliary, nonprogressive tuberculosis of one kidney. Tuberculosis of the bone marrow, seen in the lower left corner, and extensive disease in a portion of the mammary gland tissue, depicted in the lower right corner.

FIG. 11. (Lower left.) Organs of rabbit B5-1. Primary progressive generalized tuberculosis. Pretuberculous period, 8.5 months. Duration of disease, four months. Large, caseous pneumonic, primary focus in the lower lobe of the right lung, accompanied by discrete caseous foci in the draining tracheobronchial nodes, especially on the right. Scattered nodular tubercles, some of which are confluent, in both lungs. Progressive renal tuberculosis. Tuberculosis of testis, shown to the left of the left lung. Extensive tuberculosis of the costal pleura, shown in the lower left corner, and tubercles in the mucous membrane of the stomach, seen in the lower right corner.

FIG. 12. (Lower right.) Organs of rabbit B4-7. Slowly progressive tuberculosis of intermediate type. Pretuberculous period, three months. Duration of disease, seven months. There were two very small encapsulated cavities in the lower lobe of right lung which cannot be seen in the photograph. Slight caseous foci in both tracheobronchial nodes, with softening in the right. Uniformly distributed, nonprogressive, nodular, subpleural tubercles in both lungs. Caseous tubercle in the mucous membrane of the stomach, depicted below the right lung. Healing cortical tuberculosis in the kidney on the left and slowly progressive medullary disease in the kidney on the right. Tuberculosis, resulting from tubular spread to the epididymis from the kidney, shown below it. Tuberculosis of the knee joint and of the joints of the foot, shown at the bottom of the figure.

microscopic softening of the pulmonary constituent, indicating that the disease was at least of two months duration. Softening is never seen in artifically infected rabbits in less than this period. The average maximum volume of the reaction to tuberculin developed by these rabbits was 120 cmm.

The primary pulmonary focus was clearly identified in 9 of these 10 rabbits. In 6 the primary site was represented by either single or double caseous pneumonic foci. In 3 there were pus-containing encapsulated cavities. A glance at column 6 shows that the draining tracheobronchial nodes were extensively or massively involved in half of the rabbits that acquired tuberculosis. In the remaining 5 rabbits there was no involvement of the hilum nodes in some, and slight or moderate tuberculosis in others.

From these primary pulmonary foci the disease spread chiefly by lymphogenous and haematogenous pathways; canalicular spread, however, played a significant rôle in the fatal course of the disease in some of the rabbits.

In 8 of the rabbits that lived longer than two months after the onset of their disease the pleura was usually moderately involved. In column 10 are given the number and extent of the extrapulmonary vascular metastases. Besides the most usual extrapulmonary localizations of tuberculosis in the tracheobronchial nodes, the kidneys and the pleura, the disease also affected the eyes, the mammary glands, the bone marrow, the bones and joints, the brain and the meninges, and in isolated instances even the liver and muscles were tuberculous. In the same column it will be noted that from 3 to 7 organ systems were invaded by the extrapulmonary vascular metastases. This involvement ranged from moderate to extensive.

Canalicular spread to the lungs, trachea, larynx, epiglottis, pharynx, intestines and testes was prominent in some of these rabbits.

Using the criterion of the extent of involvement of the tracheobronchial nodes draining the primary focus at the portal of entry and the rate of progression of the disease in these rabbits, we find that 6 of the 10 rabbits that acquired tuberculosis developed a primary progressive generalized tuberculosis from which 2 died in four months, and 2 in six to seven months (figure 11). The other 3 died of an intercurrent infection. Three rabbits developed a slowly progressive tuberculosis intermediate in type

between primary progressive generalized tuberculosis and tuberculosis of the reinfection type with slight involvement of the hilum nodes (figure 12). Of these 2 died in seven months and one succumbed to pneumonia. One rabbit died at the end of 11.5 months of chronic ulcerative tuberculosis of the reinfection type. The duration of the disease acquired by the rabbits of the B family varied between four and 11.5 months, with an average duration of 6.9 months.

Microscopic study of the B family shows that the primary lesion was usually a caseous pneumonic focus extending peripherally by perifocal exudation of cells laden with tubercle bacilli into the surrounding alveoli. Considerable zones of intact epithelioid cells containing moderate numbers or only few bacilli bounded these primary foci. Fibrous tissue encapsulation was scanty about them. The primary focus often underwent softening, liquefaction or calcification. Bronchogenic dissemination was evident. In some rabbits there was a pronounced obliterating endarteritis; in others the vessels remained open in the centers of caseous tissue. The perivascular lymphatics were conspicuously open in most instances. There was a pronounced interstitial accumulation of mononuclears in the alveolar septa in close proximity to the tuberculous foci. The pulmonary tubercles of haematogenous origin were frequently "hard," with little caseation and few bacilli. These healed at times. Bacilli were usually rare in the epithelioid cells and especially so in the metastatic foci. Some of the lymph nodes draining the primary pulmonary lesions showed only discrete non-caseated tubercles when the lung lesions were extensively caseous. Even in the tracheobronchial nodes that had undergone complete caseation bacilli were few. Liquefaction and calcification overtook some of these lymph nodes. It is clear, therefore, that the response of the mononuclear phagocytes to the tubercle bacillus in the B family is intermediate in its effectiveness in checking the growth of the bacilli between the resistant family A on one extreme and the susceptible family F on the other extreme. Furthermore the response is not of a uniform character, being more pronounced in some and less in others. Conceivably variations in "dosage" of inhaled bacilli might account for lack of uniformity. However, the response of the A and F families, exposed simultaneously with the members of the B family, was uniform. The question of dosage is taken up in the second paper of this series.

INTERPRETATION OF RESPONSE OF FAMILIES TO NATURAL RESPIRATORY CONTAGION

Classification of Families

Duration of disease: The essential observations relative to these six families are compared in numerical form in table 7. The families may be classified from the standpoint of the duration of the disease and the degree of generalization from the pulmonary portal of entry. Under the heading of the average duration of the disease it is seen that the families naturally fell into three groups. In one extreme was the A family with a disease of 9.3 months average duration. In the other extreme were the second generation of the H family and the F and C families, with an average duration of illness of 3.6, 4.9 and 5.3 months respectively. Between these extremes were found the D family, the third and fourth generations of the H family and the B family, with average survival periods of 6.1, 6.5 and 6.9 months respectively.

Degree of generalization from the portal of entry: Approximately inversely proportional to the chronicity of the disease were the number and extent of vascular metastases, both lymphogenous and haematogenous, from the pulmonary portal of entry of the tuberculous infection. The figures listed under this heading are the average products of the number of extrapulmonary organs involved by the extent of the tuberculosis in each of these, considered numerically as follows: slight is given the arbitrary value of 1; moderate, 2; extensive, 3 and massive, 4. It will be seen that in the A family, with the most chronic disease, this product was 3.5. In the F and C families and in the second generation of the H family, surviving the shortest period, the values were 18.1, 15.0 and 14.0 respectively. In the B and D families and in the third and fourth generations of the H family, with an intermediate period of survival, the figures were 10.5, 10.3 and 9.8 respectively. Thus it is seen that a fundamental varient in the disease process of these six families is the degree to which the tuberculosis was limited to the portal of entry. In the most resistant A family there was the least amount of disease outside the pulmonary portal of entry. In the most susceptible the greatest dissemination occurred, and in the families of intermediate resistance there was an intermediate degree of extrapulmonary disease of vascular metastatic origin.

TABLE 7

Naturally acquired respiratory tuberculosis in rabbit families-summary

Family	A				F			C			H			D				B			
Generations	II	III	IV	V	II	III	IV	II	III	IV	II	III	IV	II	III	IV	V	II	III	IV	V
Number of rabbits exposed:																					
In generation	4	6	1	5	6	4	5	7	3	7	8	4	4	3	2	3	4	4	2	4	4
Total		16				15			17		8	8			12				14		
Per cent:																					
Males		50				40			59		75	62.5			50				36		
Females		50				60			41		25	37.5			50				64		
Age at beginning of exposure in months:																					
Range		2.5–12.0				4–11			2.5–13.5		2.5–6	7–12			2.5–12				2.5–12		
Median		3.5				5.3			9.5		5.0	8.5			8.0				4.7		
India ink spread:																					
Number of rabbits tested		5				5			7		—	4			4				4		
Average spread in sq. mm.		546				827			685		—	647			921				897		
Incidence of contact tbc.:																					
Number		10				12*			13		6	5			7				10		
Per cent		62.0				80.0			76.5		75.0	62.5			58.3				71.4		
Average pretuberculous period in months		5.6				6.4			5.1		6.0	3.6			5.9				6.5		
Average maximum intensity of tuberculin reaction		132				332			244		316	187			264				120		
Rabbits with pulmonary cavities:																					
Number		8				0			0		1	2			4				3		
Per cent		80				0			0		16.6	40			57.1				30		
Type of tuberculosis:																					
Primary progressive tbc.:																					
Number		0				11			13		6	2			3				6		
Per cent		0				100			100		100	40			42.9				60		
Anatomical "reinfection type":																					
Number		10				0			0		0	1			1				1		
Per cent		100				0			0		0	20			14.2				10.0		
Intermediate type:																					
Number		0				0			0		0	2			3				3		
Per cent		0				0			0		0	40			42.9				30		
Mode of pulmonary spread:																					
Bronchogenic:																					
Number		6				0			0		0	0			2				2		
Per cent		60				0			0		0	0			28.6				20		
Haematogenous:																					
Number		5				11			12		6	5			6				9		
Per cent		50				100			92		100	100			85.7				90		
Rabbits with extrapulmonary canalicular spread:																					
Number		8				11			9		5	5			7				7		
Per cent		80				100			69.2		83.3	100			100				70		
Product of number and extent of extra pulmonary vascular metastases		3.5				18.1			15.0		14.0	9.8			10.3				10.5		
Average duration of disease in months		9.3				4.9			5.3		3.6	6.5			6.1				6.9		

* One rabbit excluded from further consideration; see text.

Significant Relationships between the Relative Resistance of the Families and the Nature of Their Disease

Resistance and character of primary focus: The character of the primary focus was significantly related to the resistance of the family. In the resistant A family the primary focus progressed slowly, leading to encapsulated cavities. In the susceptible families F and C the lesion at the portal of entry was always of the fulminating, caseous pneumonic variety, and in only one rabbit of the second generation of the H family was there an excavation of the primary focus. In the families of intermediate resistance, excavation of these pulmonary foci was intermediate in incidence between these extremes; that is, the rate of progression of these pulmonary lesions was intermediate in character.

Resistance and spread to the draining tracheobronchial nodes: The spread of the disease to the lymph nodes draining the portal of entry varied according to the susceptibility of the family and paralleled the dissemination of the tuberculosis in general. This spread is the criterion for the type of disease developed by a given family. In none of the 10 rabbits of the highly resistant A family which developed the disease was there any gross tuberculosis of the tracheobronchial nodes draining the lesion at the portal of entry (figures 1 and 2). In other words the infection in these animals was always of the anatomical type considered characteristic of reinfection type disease. By contrast, in the most susceptible families, F and C and the second generation H, there was extensive caseation of these nodes in every case (figures 4, 5, 6 and 7). The tuberculosis developed by these rabbits was invariably the primary progressive type. While the number of animals observed here was relatively small, the all or none character of the variation in type of disease developed by these extremes does not require statistical analysis for its acceptance. The families of intermediate resistance B and D and the third and fourth generations of the H family were not uniform in the involvement of the nodes draining the portal of entry. Forty to 60 per cent of them developed the primary progressive type (figures 9 and 11), 10 to 20 per cent of them acquired the reinfection type, and 30 to 43 per cent of them developed an intermediate type of disease characterized by slight to moderate involvement of the tracheobronchial nodes (figures 10 and 12).

Resistance and mode of spread: The mode of dissemination from the primary pulmonary focus to the remainder of the lung and to other viscera was a function of their resistance. This spread was either vascu-

lar or canalicular. In the resistant family A bronchogenic pulmonary spread occurred in 60 per cent of the cases, while no bronchogenic dissemination was seen in the lung of the susceptible F and C families and the second generation H. In the families B and D, of intermediate resistance, bronchogenic pulmonary extension figured in a considerable number of instances. Only in the third and fourth generations of the H family, of intermediate resistance, was there no evidence of bronchogenic dissemination. This peculiarity of the latter rabbits is further discussed below.

While canalicular spread played the chief rôle in the extension of the disease in the highly resistant A family, not only in the lungs but also in the endodermal tract and the urogenital system, haematogenous dissemination also took place, though it rarely resulted in progressive lesions outside the lungs. Even in the few instances of haematogenous spread to the lungs only miliary subpleural tubercles, with little or no caseation, developed. Hence the small number and slight extent of the extraplumonary vascular metastases which characterized this family. In the susceptible families on the other hand, lymphogenous and haematogenous dissemination played the preponderant rôle in the fatal issue of their members. Canalicular spread was of subordinate importance. In the families of intermediate resistance both the vascular and the canalicular modes of extension were present.

Resistance and character of secondary lesions: The character of the secondary lesions in the lungs and in the rest of the body bore a relation to their resistance. In the highly resistant A family the secondary pulmonary foci derived, through tubular spread, from the excavated slowly progressing lesions at the portal of entry, pursued the same chronic course and became excavated in turn, until the entire lung was filled with anastamosing cavities (figure 1). This disease corresponded in its essential nature to localized ulcerative pulmonary phthisis as most commonly seen in white adults, and considered until recently as resulting solely from a reinfection. Yet these rabbits had never had contact with the disease prior to their experimental exposure.

In the susceptible families secondary lesions not only in the lungs, but in many parts of the body, resulted from blood and lymph invasion from the rapidly extending primary foci which did not become excavated. In the lung these haematogenous nodules grew apace and finally became confluent so that the entire lung was the seat of a caseous pneumonia (figures 3, 4 and 5). These, like the primary foci, extended rapidly and

did not undergo softening. Hence the large number and great extent of the extra-pulmonary vascular metastases which characterized these families. Death followed soon from a generalized progressive primary tuberculosis which had the essential characteristics of the disease seen by Borrel in the Senegalese troops. It also resembled the primary progressive type of tuberculosis seen in infants and in early childhood.

Both these types of extension, the canalicular and the vascular, were seen in the families of intermediate resistance. This would indicate that they apparently harbored determinants for both resistance and susceptibility in varying combinations. For the most resistant members of these families did not show quite the same localizing and healing capacity as the highly resistant A family, nor did the relatively susceptible members of these families of intermediate resistance show quite the same fulminating progression of the lesions as the F and C families. Hence the intermediate number and extent of the extrapulmonary vascular metastases which characterized these families. The disease in these families shared some of the characteristics of tuberculosis observed in American Negroes.

Resistance and the microscopic nature of the lesions: Microscopic analysis of the lesions in these families shows that the histological response varies according to their resistance. The lesions in the A family consisted of large stretches of intact epithelioid cells with little caseation and rare tubercle bacilli (figure 16). Only in foci of softening did the bacilli multiply (figure 17). Lesions of lymphogenous or haematogenosis origin did not prosper in the A family, but tended to resolve or to heal by cicatrization (figure 16). Even softening foci tended to become surrounded by thick layers of connective tissue (figure 14), with intact epithelioid cells still remaining at the edge of the softening mass. The mononuclears of the susceptible families characteristically swarmed with tubercle bacilli (figure 15). These epithelioid cells then underwent caseation *en masse*, killing the enmeshed blood vessels, which did not become thrombosed and lay agape in the vast caseous areas, often teeming with tubercle bacilli (figure 18). Occasionally microscopic foci of softening were found, which emptied and left behind sequestrating cavities. These were bordered not by connective tissue but by normal lung tissue, in the alveoli of which necrotic epithelioid cells swarmed with bacilli (figure 13). Progressive secondary foci occurred in which the bacilli multiplied greatly, so that in the caseous draining nodes they were present in great numbers and swarmed in the peripheral fibrolast-like cells of the abortive capsules

of these nodes. The bacilli multiplied similarly in the secondary foci of haematogenous origin in the viscera.

In the rabbits of intermediate resistance there was lack of uniformity. In some the histological picture was like that of the highly resistant A family; in others it was akin to that in the susceptible families. However no sequestrating cavities were found in these rabbits, as seen in the highly susceptible families, nor was the same degree of healing and resolution in the secondary haematogenous and lymphogenous foci of these rabbits encountered as was found in the highly resistant A family.

The H family of intermediate resistance requires additional discussion. While the large stretches of intact epithelioid cells in these rabbits contained but few bacilli there was a remarkable tendency in this family for the blood vessel walls to become involved by a deposition of epithelioid cells in the media with final desquamation of the intima and the consequent shedding of cells laden with tubercle bacilli into the lumen of the vessels. This partly explains the preponderant rôle that haematogenous dissemination played in this family. These seem to be the most significant observations relative to these families. Additional variables are listed in table 7.

Relationship between the Relative Resistance of the Families and Other Factors

Resistance and sex and age: Sex and age seemed to play no prominent rôle in the resistance to tuberculosis of the different families. It must be emphasized, however, that no rabbit was tested for its resistance to tuberculosis at less than 2.5 months. That age as such was not a significant variable in the differing response of these families is seen, for example, in families A and F. Despite the fact that the A rabbits tested were usually younger than the F rabbits, the A rabbits were unquestionably and uniformly more resistant.

Resistance, incidence of contact tuberculosis and pretuberculous period: The incidence of contact tuberculosis bore no uniform relation to the resistance of the different families; this incidence ranged between approximately 60 and 80 per cent in all the families. Nor was there any constant relation between susceptibility to tuberculosis and the pretuberculous period. The interval elapsing between the beginning of exposure and the onset of tuberculosis in all the families ranged approximately between four and six months.

Resistance and spread of India ink in the skin: As noted above the

spread of India ink in the skin may bear a relation to resistance to tuberculosis. Unfortunately, as was discovered, there was considerable individual variation in this respect in the members of any given family. There is also a sex limited variation in this property of the skin. Therefore the observations were too few in number for statistical significance. Nevertheless it is interesting that the two families, A and F, which differed most constantly and sharply in their resistance to tuberculosis also differed constantly in their capacity to spread India ink in the skin. This was true in all generations tested and not only in those listed in this experiment. There was thus a parallel between the spread of a nonspecific substance in the skin and the spread of tuberculosis in the tissues of these two highly uniform and highly contrasting families. Both the dye and the tuberculosis spread widely in the susceptible F family. In the A family both tended to become localized at the site of introduction of the agent. However, there was no constant correlation between this spread and the resistance to tuberculosis in all the families. Furthermore, while resistance to tuberculosis was relatively constant through successive inbred generations, the permeation of the skin by this particulate dye and also by diffuse rabbit haemoglobin was not constant. Thus in the fifth generation the B rabbits spread the dye very widely, whereas in previous generations the B rabbits had localized these dyes sharply. Nevertheless the resistance of this family was the same in all these generations. The D family also showed similar discrepancies.

Resistance and intensity of tuberculin sensitivity: Another interesting variation in these families, which was again incompletely related to resistance and of limited significance because of the small numbers of animals involved, was the average maximum intensity of the tuberculin reaction developed by these rabbit families during the course of their naturally acquired disease. The highly resistant A family developed a low sensitivity to tuberculin; the susceptible families, F, C and the second generation H, on the other hand, became definitely more sensitive. Furthermore, the third and fourth generations of the H family, of definitely greater resistance than the second generation of this family, developed a considerably lower average maximum tuberculin sensitivity. This sensitivity was intermediate in degree between the highly resistant A family on the one hand and the highly susceptible families F, C and the second generation H rabbits on the other. It will be remembered that the sensitivity to tuberculin tends to be higher in human beings of apparently greater susceptibility to tuberculosis like the American or

South African Negro than that in the presumably more resistant whites. The observations on these families corresponded therefore to similar differences in man. However, this correlation was incomplete in families D and B. The family D, of considerable resistance to tuberculosis, showed the same relative sensitivity to tuberculin as the highly susceptible C family, and the B family showed a lower allergic sensitivity than the members of the A family, of unquestionably higher resistance to tuberculosis. These observations served as a starting point for further studies directed in an attempt to define to some extent those factors in the constitutional character which make for resistance and susceptibility. This question is considered in a subsequent paper.

Resistance, genetic constitution and environment: It is clear, however, at this point that the genetic constitution of a rabbit plays a large rôle in his resistance to naturally acquired tuberculosis. For the only known variable of these families of characteristic inherited resistance to tuberculosis is their forbears. All the environmental variables such as feeding, rearing and freedom from contact with disease, were constant not only in the rabbits tested at a given time, but also in their ancestors for as many as four generations, a condition which can never be approached with human material.

Resistance and color markings: It has been reported that rabbits of certain color markings are more resistant to tuberculosis than those with others (23). In this study the color markings of the rabbits played no significant rôle in their resistance to the disease. Thus the A and B families were all either albinos or one of three varieties of the English markings: (1) fully colored or *en en*, the homozygous recessive type, (2) the heterozygous or exhibition English type, *En en*, or (3) the homozygous dominant combination, *En En*, the Chaplin type; yet in generation after inbred generation the B family was definitely less resistant to the disease than the A family of similar color markings. Furthermore, as already pointed out in the analysis of the pedigree of these families, the only difference between the A and B families was the different original mother of the A family, A1-2, the albino doe with suppressed exhibition English markings.

That color markings are of little significance in the resistance of rabbits to tuberculosis is illustrated by comparing the highly resistant A family and the highly susceptible F family. In the fourth generation of the F family most of the rabbits had English markings of the Chaplin type. The genetic analysis furnished by breeding these rabbits shows that these

were of the constitution *En du*^{d} or *En du*^{w}, where the *du* or Dutch quality is recessive to the En or English genes. These Chaplins of the F family were indistinguishable from chaplins derived from the A or B families. If resistance to tuberculosis were associated with the genes determining English color distribution, the F family should be resistant, for in many rabbits of this family the English markings are dominant. Yet the F's, whether they were Dutch in color or English, were equally, greatly and constantly more susceptible than the A rabbits and even the B rabbits of similar markings.

Similar statements can be made in regard to the second generation of the H family, on the one hand, a family of great susceptibility to the disease, and the third and fourth generations of the same family. Their color markings were the same; yet their resistance was quite different.

Resistance and the genetic history of the families: Analysis of the ancestry of the families through their entire known genetic history and not only that obtained in our laboratory, shows that similar parentage accounts for similar reaction patterns to tuberculosis, and that the degree of uniformity of the response is a function of the degree of inbreeding of the forbears. Thus the A and B families had a common ancestor, A1-1, and the father of the third inbred generation of the B family was a member of the A family. Accordingly the resistance of the B family is closely related to that of the A. Two rabbits, 1 and 27, repeatedly inbred, were almost the sole ancestors of the F family. This family was uniformly and highly susceptible. The same two rabbits were also the preponderant forbears of the C family. Accordingly the vast majority of them were as susceptible as the F family. However, the degree of inbreeding of these two rabbits was much less in the forbears of the C family. Accordingly, a small proportion of C's reacted differently to the disease. Similar principles have been shown to hold for the remaining families.

SUMMARY

Six inbred rabbit families, A, B, C, D, F, and H were exposed to natural respiratory contagion of tuberculosis through 4 to 5 successive inbred generations. From the standpoint of the duration of the acquired disease and the degree of generalization from the pulmonary portal of entry, the families fell into three groups. The A family was most resistant, with the most chronic disease and the least generalization from the portal of entry. The F and C families and the second generation of the H family

were most susceptible, with disease of the shortest duration and the greatest degree of generalization. The B and D families and the third and fourth generations of the H family were of intermediate resistance with disease of intermediate duration and intermediate degree of generalization.

The primary focus in the lungs of the resistant A family usually progressed slowly and appeared at death as a well encapsulated cavity. The draining tracheobronchial nodes were uniformly free from gross tuberculosis. The disease was always anatomically of the type commonly considered characteristic of reinfection. It progressed in the lung by canalicular spread. Tubular spread to the endodermal tract from ulcerated foci in the lungs and to the urogenital system from the kidneys also caused progressive disease. The secondary foci became excavated in turn and frequently the lungs were honeycombed by numerous cavities. Haematogenous spread caused few if any progressive lesions, even when this occurred in the lungs.

The primary lesions in the members of the susceptible F and C families and the second generation of the H family were uniformly rapidly progressive, caseous, pneumonic foci with little or no encapsulation and no true cavity formation. Sequestrating cavities without fibrous tissue response were seen in these families. The draining tracheobronchial nodes were invariably massively caseous. The disease was always of the anatomical type commonly considered characteristic of progressive primary infection. The disease spread to the lungs and to the rest of the body by haematogenous routes in particular. The haematogenous lesions progressed rapidly wherever focalized. In the lung they gave rise to miliary tuberculosis, or to confluent nodular caseous pneumonia. In the viscera they resulted in large nodular caseous lesions. Canalicular spread to the lungs never occurred.

The primary focus in the rabbits of intermediate resistance progressed in a variable manner. In some it was almost, though not quite as fulminating as in the susceptible families. In others encapsulated cavities were found at autopsy. Likewise the draining tracheobronchial nodes were often extensively caseous. In others there was little or no involvement of these nodes. The anatomical character of the disease was therefore mixed. In some animals it was of the progressive primary type, in others of an intermediate character, and in a few of the type commonly considered characteristic of reinfection. Both haematogenous and tubu-

lar dissemination were active in the spread of the disease to the lungs and the remaining organs in these rabbits. At times the one, at times the other mode was predominant.

Microscopic analysis of the lesions in these families suggests that the mononuclears of the A family rapidly acquired an effective capacity to inhibit the growth of the bacilli. Bacilli were rarely found in the cells. Only in foci of softening did they multiply. In the susceptible families, on the other hand, these cells swarmed with tubercle bacilli. The response of the mononuclears of the families of intermediate resistance was again variable.

Analysis of the forebears of these families indicates that similar response to tuberculosis is a function of common ancestry. The environmental conditions of these rabbits were always uniform. The only variable in these families was the genetic constitution of the rabbits. Color, sex and age, beyond 2.5 months, were not significant factors in resistance to tuberculosis. There was some incomplete relationship between the spread of India ink in the skin and their resistance to tuberculosis. Likewise the tuberculin sensitivity of the most resistant families tended to be lower than that of the susceptible families, but this relationship was again not constant. Further data relative to these rabbits are presented in subsequent papers.

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II. INHERITED NATIVE RESISTANCE AND ARTIFICIAL TUBERCULOUS INFECTION

It has been shown in the preceding paper that the resistance of rabbits to naturally acquired respiratory tuberculosis is largely controlled by the genetic constitution of the animal. The mode of acquisition of the disease by these rabbit families closely simulated the natural manner of infection in man. Moreover, types of tuberculosis similar to those seen in different human ethnic groups were developed by the three family groups studied, viz., the highly resistant family with its localized, chronic ulcerative phthisis of the reinfection type, the highly susceptible families with their rapidly progressive primary generalized disease and the families of intermediate resistance with a tuberculosis of a character intermediate between these extremes. However, there is one fundamental shortcoming to the procedure as used: little can be said of the dose of tubercle bacilli that actually penetrated the lungs of the members of the different families, nor is anything known of the number of such inhalations or of the intervals between them. Therefore it is desirable to determine whether similar differences in resistance to tuberculosis will also obtain when uniform quantities of the same suspension of tubercle bacilli are introduced simultaneously into representatives of the different families.

RESISTANCE TO INOCULATION OF STANDARD DOSES OF TUBERCLE BACILLI

In table 1 are recorded several determinations of the varying survival of different families inoculated with standard quantities of the bovine strain, Ravenel. Families A and B of the third generation, and family H of the second generation were injected with 0.2 mg. of this microorganism intracutaneously.

The order of resistance of these three families as measured by their average survival after this standard inoculation was the same as that found by natural respiratory contagion. The A family was most resistant, surviving 276 $\pm$ 25 days; the B rabbits were of intermediate resistance, surviving 165 $\pm$ 30 days; and the H rabbits were the most susceptible, surviving 124 $\pm$ 21 days. The difference in survival between

the A and H rabbits was statistically significant when analyzed according to the "Student" t distribution curve, P being 99.99. The differ-

TABLE 1

Resistance of rabbit families to inoculation of standard doses of bovine tubercle bacilli

0.2 MG. INTRACUTANEOUSLY		0.001 MG. PER KILO INTRAVENOUSLY		0.000,1 MG. PER KILO INTRAVENOUSLY	
Rabbit number, generation and family	Survival time in days	Rabbit number, generation and family	Survival time in days	Rabbit number, generation and family	Survival time in days
A3-9	291			A5=1	199
A3-11	296				
A3-12	241*				
Average.....	276 ±25				
B3-5	176	B3=12	125*	B3-12	133
B3-6	123*	B3=13	196	B3-9	193
B3-7	196	B3=16	170		
		B3=15	159		
Average.....	165 ±30		162 ±25		
H2-19	123	H2-29	79	H2-12	133
H2-22	108	H2-31	73	H2-14	109
H2-23	133	H2-26	78	H2-25	112
H2-24	134	H2-32	82	H3-10	108
Average.....	124 ±21		78 ±3		115 ±10
				C2-17	77
				C3-22	101
				C3-23	85
				C3-24	91
				C3-25	94
Average.....					89 ±8
				F3-14	93
				F3-23	83
				F3-25	75
				F3-26	85
Average.....					84 ±6

* Died of intercurrent disease.

ence in the average survival between the A and B rabbits was also statistically significant, P being 99.4. The difference between the B and H rabbits in this test was not of equal statistical significance, P being 94.9.

In the second test, however, where the same generations of the B and H families were inoculated intravenously with 0.001 mg. Ravenel strain bacilli per kilogram of body weight, the B family was unquestionably more resistant than the second generation of the H family. The B rabbits survived 162 ± 25 days and the H rabbits, 78 ± 3 days. The difference in the survival between these two families was statistically significant, P being 99.99.

In a third test, three rabbits of the second generation and one of the third generation of the H family were compared with the same generations of the F and C families in the same proportions. One A rabbit of the fifth generation and 2 B rabbits of the third generation were included in this determination. All of them received 0.000,1 mg. of Ravenel strain bacilli per kilogram. It is clearly seen that the C and F rabbits were of equal susceptibility, surviving 89 ± 8 and 84 ± 6 days respectively. The second generation of the H family survived a somewhat longer period, 115 ± 10 days, and the difference between the H family on the one hand, the F and C families on the other, slight though it was, was nevertheless statistically significant, P being 99.7 for both. The survival time of the single A rabbit and of the B rabbits was within their position of relative resistance as determined in the remaining tests of this series.

Thus it is seen, on the basis of statistically reliable differences in the survival of rabbits of the A, B, C, and F families and of the second generation of the H family, following standard inoculations of virulent bovine type tubercle bacilli, that the order of resistance for these families was the same as that determined by natural exposure. The A family was of highest resistance, the F and C families and the second generation H family were of greatest susceptibility, and the B family was of intermediate resistance.

Essentially similar data for the rabbits of the D family and for the third and fourth generations of the H family, presented in the subsequent paper, place these families in the same relative category of resistance as determined by natural exposure.

RESISTANCE TO QUANTATIVE NATURAL RESPIRATORY CONTAGION

While these artificial methods of infection with standard doses demonstrated the same differences in resistance of the rabbit families observed after natural respiratory contagion, the disease thus acquired resembled but slightly the various types of tuberculosis developed by these families

on natural infection. In an effort to overcome the shortcomings of uncontrolled dosage in natural contagion, an apparatus for the quantitative natural respiratory contagion devised by Wells was used on some of these families. The experiments have been reported in detail elsewhere by Wells and the author (1).

Briefly the method was as follows. Rabbits whose relative natural resistance was to be determined were placed simultaneously in a large airtight bell jar. A fine suspension of the same highly virulent bovine strain, Ravenel, as used heretofore, was atomized with compressed air in a specially constructed flask. A uniform flow of minute droplet nuclei, containing the bacilli and visible only in the Tyndall effect, was delivered from the mouth of the flask into a long duct. The contaminated air from this duct was drawn into the bell jar by the draught action of a hot flame at the bottom of a chimney connected with the outlet of the bell jar. The chimney acted both as a suction apparatus and as an incinerator for the tubercle bacilli. By means of the Wells air centrifuge known volumes of air were sampled for the number of tubercle bacilli which they contained. This was accomplished by culturing glycerine broth in which the particles suspended in a known volume of air were incorporated by the centrifugal action of the machine. The number of colonies cultured from such air samples represented the number of units of tubercle bacilli suspended therein, without reference to the number of individual bacilli originally present in each unit. In 12 rabbits belonging to these families the amount of air breathed in a unit time under these conditions was determined in a Murphy respirator with the use of a Krogh spirometer (2). Since the concentration of the tubercle bacilli in the air was known, and since the total volume of air breathed by an average rabbit had been experimentally determined, the approximate number of tubercle bacilli inhaled could be estimated.

In table 2, reproduced here from the paper referred to above, data are presented on the fate of rabbits of some of the families thus exposed. It will be seen in the case of the three rabbits killed on the day folllwing exposure that the number of bacilli cultured from their lungs corresponded closely to the number of bacillary units estimated to have been inhaled by these rabbits. The greater the estimated number of bacilli inhaled the more numerous were the resulting caseous nodules in the lungs. They were innumerable with a dose of 1,000 or more bacillary units and were in small numbers when the estimated dose was about twenty. Furthermore the duration of the preallergic period, the survival time of the

TABLE 2

The fate of rabbits descended from different families, following inhalation of varying quantities of tubercle bacilli

EXPERIMENT NUMBER	RABBIT NUMBER, GENERATION AND FAMILY	NUMBER OF ESTIMATED TUBERCLE BACILLI INHALED	NUMBER OF TUBERCLE BACILLI CULTURED FROM LUNG	PREALLERGIC PERIOD IN DAYS	SURVIVAL TIME IN DAYS	TYPE OF TUBERCULOSIS
1	H4-14	6,818	5,452	—	*	—
	H5-19	6,818		15	38	Massive nodular caseous pneumonia with tbc. of tracheobronchial nodes
2	A5-27	2,772		15	43	Nodular caseous pneumonia
	A5-28	2,772		no allergy	43	Nodular caseous pneumonia
3	F4-34	2,100		15	46	Nodular caseous pneumonia
	F4-39	2,100		15	34	Nodular caseous pneumonia
4	C4S-23	1,430	1,210		*	
	C4S-22	1,430		20	41	Nodular caseous pneumonia
5	C4S-5	676		13	36	Nodular caseous pneumonia
	C4S-4	676		21	160	Nodular caseous pneumonia with calcification; tbc. of tracheobronchial nodes and vascular metastases to 13 organ systems
6	A5-29	585		20	100	Massive ulcerative caseous pneumonia with tbc. of tracheobronchial nodes; slight intestinal and renal tbc.
	H5-9	585		20	70	Massive caseous pneumonia with beginning softening; extensive tbc. of tracheobronchial nodes and slight renal tbc.
7	A5-39	550		14	71	Caseous pneumonia with perifocal inflammation; slight tbc. of tracheobronchial nodes
	H5-2	550	660		*	
8	A5-36	120		35	174	Extensive ulcerative pulmonary tbc.; slight fibrous tbc. of tracheobronchial nodes; tbc. of salivary glands; moderate renal tbc.; tbc. of larynx and one joint

* Killed on day following exposure.

TABLE 2—*Concluded*

EXPERIMENT NUMBER	RABBIT NUMBER, GENERATION AND FAMILY	NUMBER OF ESTIMATED TUBERCLE BACILLI INHALED	NUMBER OF TUBERCLE BACILLI CULTURED FROM LUNG	PREALLERGIC PERIOD IN DAYS	SURVIVAL TIME IN DAYS	TYPE OF TUBERCULOSIS
8	F4-45	120		28	114	Caseous pneumonia; extensive caseous tbc. of tracheobronchial nodes; extensive caseous renal tbc.; tbc. of pleura, intestines, heart and skeletal muscles
9	A5•33	94		42	140	Ulcerative phthisis with numerous pulmonary cavities; tracheobronchial nodes normal; slight renal tbc.
10	A5•38	27		27	161	Ulcerative phthisis with numerous pulmonary cavities; tracheobronchial nodes normal; minimal renal tbc.
	F4-52	27		27	102	Caseous pneumonia; extensive caseation of tracheobronchial nodes; massive renal tbc.
11	B5-23	23		55	110	Nine pulmonary foci, some ulcerated; slight tbc. of tracheobronchial nodes; fatal intercurrent enterocolitis

rabbits and the type of disease developed by them, were to a considerable extent influenced by the dose of inhaled bacilli estimated, as indicated in these experiments. Irrespective of the natural resistance of the family, the larger the dose inhaled the shorter was the preallergic period, the shorter the survival time of the animals and the more fulminating the ensuing disease. Therefore a parallel has been established between the graded effects of inhaling increasing doses of tubercle bacilli and similar graded effects of introducing increasing "doses" of tubercle bacilli parenterally, viz. intravenously. Additional data bearing on the reliability of this method of quantitative natural respiratory contagion will be found in the detailed report.

From the standpoint of natural inherited resistance to tuberculosis the following observations are of significance. If the dose of bacilli inhaled

was about 1,000 or more, it mattered little whether the animal exposed belonged to the A, F, C or H families. All died in five to six weeks of a nodular caseous pneumonia with their lungs almost completely consolidated by innumerable closely lying, caseous lesions. Though these tended to be somewhat less numerous and less extensively caseous in the members of the A family than in members of the other families, there was little else which was conspicuously different, in the gross, between them. The disease thus acquired bore little resemblance to the natural disease in any family. It resulted from a sudden simultaneous seeding of both lungs with numerous bacilli. Hence the ensuing lesions represented great numbers of primary pulmonary foci each one of which was presumably the consequence of one or more units of bacilli trapped in a terminal air passage. So numerous were these primary foci that not enough time elapsed for the extension of the tuberculosis even to the draining nodes. Nor was the disease of sufficient duration to permit its spread to other viscera by blood stream invasion. Death resulted from simple consolidation of the lungs and respiratory failure.

It has been shown in the preceding paper that in the naturally acquired disease in any family there was usually a single primary focus in the lung from which the disease extended just as in the natural disease of man. It is obvious therefore that little can be learned of the natural resistance of an animal when its entire lungs are thus suddenly flooded with large numbers of bacilli.

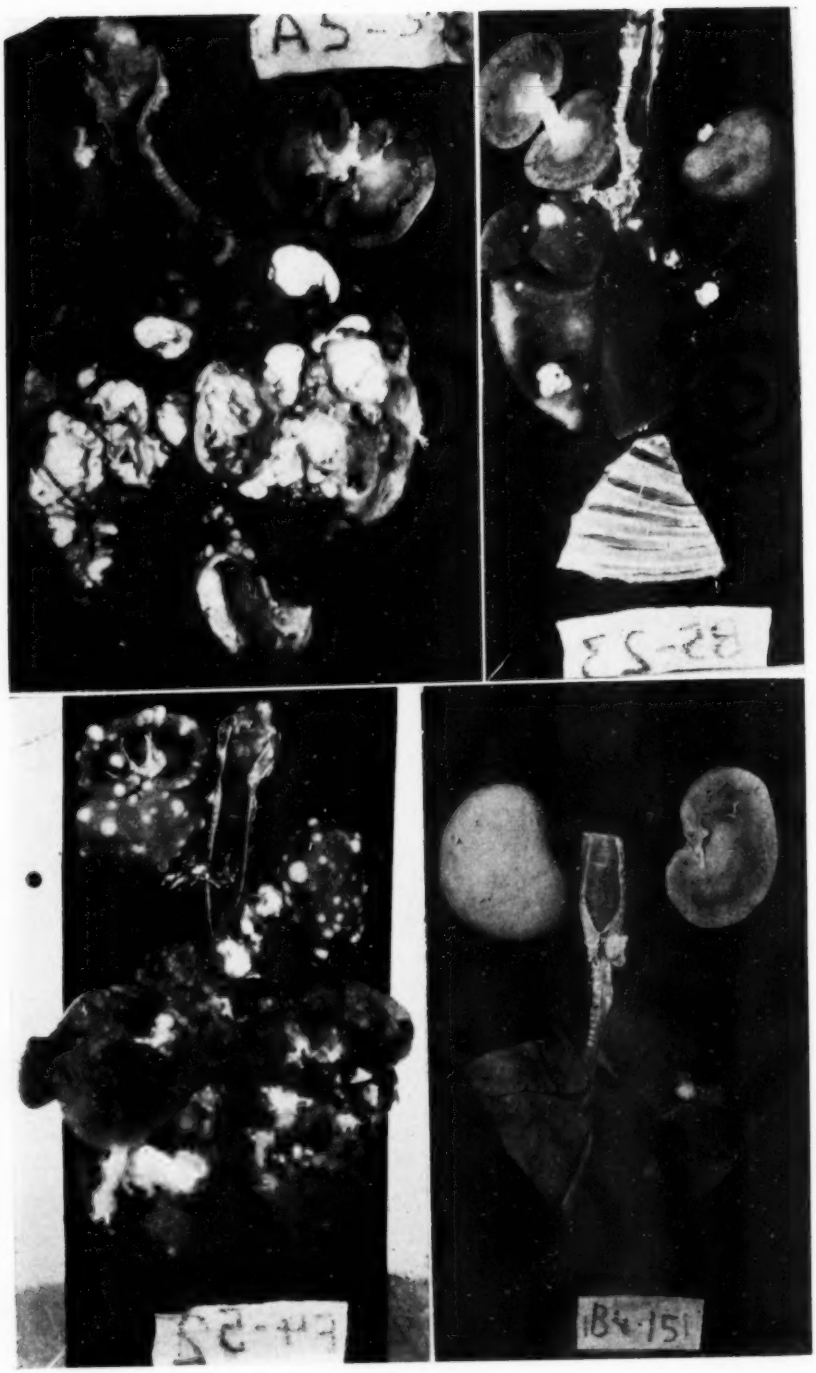
With doses of five to six hundred bacilli inhaled at one sitting the natural resistance of the different families began to come to the fore, although not uniformly so. Thus A5=29 and H5=9 were exposed simultaneously to an inhalation of approximately 600 units of tubercle bacilli. The A rabbit died in one hundred days of ulcerative caseous pneumonia with well walled-off cavities, slight tuberculosis of the draining nodes and minimal renal and intestinal tuberculosis. The H rabbit, on the other hand, died a month earlier with massive caseous pneumonia, with beginning softening without encapsulation, with extensive caseation of the tracheobronchial nodes and slight renal disease. It is clear that with this dose the A rabbit exhibited a greater resistance than the H rabbit. The remaining rabbits in this group are compared to better advantage later.

It was when the dose of estimated inhaled bacilli was below 100 that the natural resistance of the families really asserted itself. Thus A5=38 and F4-52 both inhaled approximately 30 units of tubercle bacilli. F4-52

(figure 2) died in one hundred-two days with confluent areas of caseous pneumonia, extensive caseation of the tracheobronchial nodes and massive tuberculosis of the kidneys. The tuberculosis corresponded in its essentials to the disease as naturally acquired by members of this family when exposed to tuberculous cage mates. There was one exception, however; instead of a single primary focus there were confluent areas of caseous pneumonia apparently not of haematogenous origin and really multiple primary foci. A5=38, on the other hand, died in one hundred sixty-one days from a disease strictly localized to the portal of entry, the lungs (figure 1). The draining tracheobronchial nodes were entirely normal and there was only minimal renal disease. This ulcerative pulmonary phthisis of the "reinfection" type corresponded to the disease developed by members of this family when exposed to tuberculous cage mates, as reported in the previous paper. Essentially similar differences were shown in the rabbits F4-45 on the one hand and A5=33 and A5=36 on the other, which inhaled between 100 and 120 units of tubercle bacilli. The susceptible F died of primary progressive generalized tuberculosis in 114 days. The resistant A rabbits died in 140 and 174 days respectively, of ulcerative pulmonary phthisis of the "reinfection" type. The only apparent differences between the natural disease and that acquired by quantitative inhalation in the A family were the small size of the individual cavities in the natural infection in contrast to the huge cavities in the artificial infection and the relatively greater difference in duration of the disease between the A and F families in the natural infection as contrasted with that difference in the artificial infection.

Thus as a result of a single exposure to small numbers of tubercle bacilli, not only could the relative resistance of these rabbit families be demonstrated but the essentials of the natural disease characteristic for two of these families, at least, could be reproduced.

At this point it is advantageous to return to the two rabbits of the C family, in experiment 5. C4 S-4 and C4 S-5 were exposed simultaneously to approximately 700 units of tubercle bacilli. The latter died in thirty-six days of nodular caseous pneumonia, a disease as fulminating as that of other rabbits of the same family which received larger doses. C4 S-4, however, did not die until one hundred sixty days after the inhalation (See figure 6 in preceding paper). It is noteworthy that while the progression of the lesions, particularly in the lung, was slow, as evidenced by their calcification, the tuberculosis was still of the primary type with caseation of the draining nodes and extensive metastases to numerous



FIGS. 1-4

organs. In contrast, in A5=38, which survived the same period, the disease was strictly localized to the lungs and the animal died of chronic ulcerative pulmonary phthisis of the "reinfection type" (figure 1). It will be remembered from the preceding paper that, while the majority of the C rabbits were highly susceptible, an occasional member of this family was found in which the lesions progressed slowly though the disease had all the earmarks of a primary generalized tuberculosis, just as in C4 S-4).

In the eleventh experiment, a rabbit of the B family was estimated to have inhaled approximately 20 units of tubercle bacilli. In the lungs of this rabbit, which died of enterocolitis, 9 separate nodules were found, some of which were excavated, with slight tuberculosis of the draining tracheobronchial nodes (figure 3). This is to be contrasted with B4-15, a member of the B family which acquired the disease by exposure to tuberculous cage mates and died of an intercurrent pneumonia (figure 4). In its lungs there was present a single caseous pneumonic focus

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FIG. 1. (Upper left.) Organs of rabbit A5=38. Rabbit inhaled an estimated dose of 27 units of tubercle bacilli at a single exposure. Preallergic period, twenty-seven days. Duration of disease, one hundred and sixty-one days. Strictly localized ulcerative pulmonary phthisis analogous to the reinfection type. The draining tracheobronchial nodes are somewhat enlarged but grossly and microscopically free from tuberculosis. Three large cavities may be seen in the right lung, and a smaller one may be detected in the left. The disease exhibits the essential characteristic of the infection contracted by A3-15 through natural exposure to tuberculous cage mates. The photograph of the organs of the latter rabbit may be found in the first figure of the preceding paper.

FIG. 2. (Lower left.) Organs of rabbit F4-52. This rabbit was exposed simultaneously with rabbit A5=38 shown in figure 1, and inhaled an estimated dose of 27 units of tubercle bacilli. Preallergic period, twenty-seven days. Duration of disease, one hundred and two days. Primary progressive generalized tuberculosis. Multiple foci of confluent caseous pneumonia with extensive enlargement and caseation of draining tracheobronchial nodes. Extensive, progressive tuberculosis of both kidneys. The disease has the essential characteristics of the infection contracted by F4-33 through natural exposure to tuberculous cage mates. The photograph of the organs of the latter rabbit may be found in figure 3 of the preceding paper.

FIG. 3. (Upper right.) Organs of rabbit B5-23 which inhaled an estimated dose of 23 units of tubercle bacilli. Nine primary pulmonary foci, some of which were excavated, could be identified in its lungs. Five of these may be seen in the photograph. There was slight involvement of the tracheobronchial nodes and of the costal pleura. Rabbit died of intercurrent enterocolitis on the one hundred and tenth day following exposure.

FIG. 4. (Lower right.) Organs of rabbit B4-15 which contracted tuberculosis naturally by exposure to tuberculous cage mates as recorded in the preceding paper. Rabbit died of pneumonia 1.3 months after development of a positive tuberculin reaction. There is a single, primary, pulmonary focus in the middle of the right lung accompanied by enlargement and slight caseation of the homolateral draining tracheobronchial node.

with slight tuberculosis of the homolateral tracheobronchial nodes. These two rabbits illustrate the inference drawn from this study that in naturally acquired tuberculosis the disease usually originates from a single "unit" of tubercle bacilli that is trapped in the alveoli. For in all the tests thus far made with the inhalation of as few as 20 units of bacilli at least 9 pulmonary nodules resulted.

SUMMARY AND DISCUSSION

It has been shown in this paper that the relative resistance of the families A, B, C, F and the second generation H to standard doses of virulent bovine type tubercle bacilli, parenterally introduced, was the same as that found in these families when they acquired the disease by natural respiratory contagion. The A family was of highest resistance, the F, C and second generation H were of lowest resistance and family B was of intermediate resistance to tuberculosis.

By exposing some of these families in an apparatus which permits one to estimate with relative accuracy the number of units of tubercle bacilli inhaled at a single sitting, it was found that when the dose of bacillary units inhaled exceeded 1,000, there was little difference in the fate of the rabbits irrespective from what family they originated. All of them died from a fulminating disease with innumerable caseous pneumonic nodules which were in fact so many primary pulmonary foci. The disease thus acquired, however, bore no resemblance to naturally acquired tuberculosis. If, however, rabbits of the A and F families were caused to inhale numbers of bacillary units ranging from 20 to 100, the disease acquired by these two families was essentially like the tuberculosis developed by these same families when exposed to tuberculous cage mates in the experiments on natural respiratory contagion recorded in the preceding paper. The F rabbits developed a disease originating in multiple primary foci in the lungs which soon spread to the draining tracheobronchial lymph nodes, which became extensively caseous. They died shortly of a primary progressive generalized tuberculosis with caseous pneumonia and destructive lesions of haematogenous origin in the kidneys. The A family, on the other hand, simultaneously exposed, at a single sitting, to the same number of bacillary units developed a disease which remained localized at the portal of entry. The animals died after a more prolonged illness from localized ulcerative pulmonary phthisis of the "reinfection" type without involvement of the draining nodes and with little or no spread to the internal organs.

However, there still remain some differences between the naturally acquired disease and that of quantitative natural respiratory contagion. There was a greater difference between the resistant and the susceptible families in the duration of the disease after the natural infection than after artificial infection. In the former the origin of the pulmonary disease was in a single primary focus. In the latter, even when as few as 20 bacillary units had been inhaled, at least 9 primary foci resulted. This lends strength to the provisional inference that in the naturally acquired disease of rabbits and also of man a single unit of tubercle bacilli is probably responsible for the primary focus.

The results also showed that it is not necessary to assume that the reason for the "reinfection" type of tuberculosis uniformly developed by the members of the highly resistant A family was immunization with subinfectious doses of inhaled tubercle bacilli. For it has been shown in these experiments that after a single exposure rabbits of the A family developed chronic ulcerative phthisis without involvement of the tracheobronchial nodes, i.e., the type of tuberculosis characteristic of reinfection. The only difference in the disease thus acquired and the natural infection was that the cavities developed by these rabbits were huge in comparison with the smaller anastomosing cavities found in the natural disease. Furthermore the survival time of the rabbits artificially infected was apparently also less. Both of these differences are attributable to the apparently larger numbers of tubercle bacilli inhaled by these rabbits as compared with those which acquired the disease by exposure to tuberculous cage mates.

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III. EXPERIMENTAL STUDY OF CERTAIN FACTORS RELATED TO HEREDITARY CONSTITUTIONAL RESISTANCE TO TUBERCULOSIS

MAX B. LURIE AND PETER ZAPPASODI

In the first paper of this series it was shown that the six rabbit families under investigation exhibit varying and characteristic resistance to naturally acquired respiratory tuberculosis which is determined by the genetic constitution of each of these families. Analysis of the type and progress of the disease developed indicates that the fundamental variant in the disease process in these families is the degree to which the infection is limited to the portal of entry of the bacillus. This in turn is apparently controlled by the capacity of the mononuclear cells at the portal of entry primarily, but also in the secondary foci, to inhibit the the growth of the bacilli. In the second paper of this series evidence has been presented for four of these families indicating that their characteristic inherited resistance can be demonstrated also by the inoculation of standard quantities of tubercle bacilli. For the two remaining families similar evidence will be presented shortly. It was shown in the same study that the characteristic resistance of some of these families can be demonstrated also by exposing them to the natural inhalation of known small numbers of tubercle bacilli at a single sitting. Furthermore, the fundamental difference in the disease type, as developed on natural respiratory contagion by a resistant and a susceptible family, can be reproduced in its essentials by this quantitative method.

The purpose of the present study was to obtain further information on the anatomical and physiological constitutional qualities which control resistance and susceptibility to tuberculosis. The strikingly localized character of the naturally acquired disease in the highly resistant A family on the one hand, and the rapidly spreading character of the same infection in the highly susceptible F family on the other hand, suggested the possibility that a nonspecific permeability of the tissues may be a function in resistance to tuberculosis. Again the greater degree of allergic sensitivity developed by the more susceptible families as compared with the more resistant rabbits during the course of their natural infec-

tion, pointed to the possibility that the native capacity of a family to become hypersensitive to the tubercle bacillus may play a rôle in its resistance. Finally, the fulminating progress of the primary lesion in the lungs of the susceptible families in contrast with the slowly progressing encapsulating and excavating primary foci in the lungs of the most resistant family, suggested that the characters which determine the rate and intensity of development of local immunity are dominating factors in the inherited resistance of these families to tuberculosis. Furthermore, Lewis and Loomis (1) studied the local lesion that developed at the site of an intracutaneous inoculation of tubercle bacilli in inbred guinea pig families of varying inherited natural resistance to tuberculosis. They found a general, though imperfect, parallel between the natural resistance of the family and the efficacy of the healing process at the portal of entry in the skin.

MATERIALS AND METHODS

The following study was undertaken:

Four to 6 members of the fourth or fifth inbred generations of the A, B, C, D, F, and H families were obtained. These were mostly males and usually between seven and eleven months of age. The few rabbits which did not fall into these categories are indicated in the accompanying table 1. All 28 rabbits in the experiment received intracutaneously in the flank 0.5 cc. of a 1:5 dilution in saline of autoclaved India ink. On the next day the spread of the dye was determined as described in the first paper of this series. All of the rabbits were then subjected to a course of intracutaneous inoculations of the same quantities of heat-killed Ravenel bovine type bacilli at the same intervals and in the same varying relative location at each interval. The volume of the nodule resulting from each successive inoculation was determined as described in the first paper.

The nodule resulting from the third injection of heat killed tubercle bacilli was removed for microscopic analysis two days after the injection in order to see whether, at this stage, there was any observable difference in the structure and cellular composition of the lesion in different families.

The volume of inflammation resulting from successive intracutaneous injections of 0.1 cc. of 10 per cent Old Tuberculin was determined at the same intervals during the course of treatment in all these six familes. The development of allergic sensitivity in these rabbits, resulting as it did from simultaneous injections of equal amounts of nonmultiplying dead bacilli, must be regarded as a measure of the innate allergic irritability, or the capacity of the tissues of the different families to become hypersensitive to tuberculin. This

allergic sensitivity differs from that developed by animals infected with living tubercle bacilli, for obviously the rate of multiplication of the bacilli in different families may be different, and hence the ensuing allergy may differ accordingly, with no relation to the innate responsiveness of the tissues of different families to this irritant.

On the eighth and fifty-fifth day following the beginning of treatment with heat-killed tubercle bacilli the agglutinin titre of the serum of each member of each family was determined by the resuspension technique (2). This titre is a measure of the innate capacity of the different families to produce antibodies in response to the same antigenic stimulus.

One month after the last injection of heat-killed tubercle bacilli all of these rabbits received intracutaneously in the left chest wall 0.2 mg. of the same suspension of living virulent Ravenel bovine type tubercle bacilli in a volume of 0.2 cc. The development of the lesion at this portal of entry, as well as the changes in the draining axillary nodes, were followed throughout the course of the disease.

On the sixty-fifth day after the virulent infection, India ink was again injected into the skin, and its spread was measured in the same manner as above described in order to determine the effect which a virulent infection exercised on this property of the skin in different families.

The volume of the local lesion was determined at weekly or biweekly intervals until the death of these animals, at which time they were carefully autopsied. The volume of the local lesion and its microscopic structure were determined. The volume of the tuberculous draining nodes was recorded and the extent of the disease beyond these nodes was estimated numerically by adding the figures obtained from the following scheme: A slight tuberculous involvement of any organ was arbitrarily assigned the number 1 and moderate, extensive and massive disease of any organ or system were given the values 2, 3 and 4 respectively. With these observations as a basis, the disease developed by each of these rabbits was classified. All of the data for each of the rabbits of the 6 families were now correlated with the resistance of the same individuals to tuberculosis as measured by their survival time after this standard inoculation following the same preliminary treatment.

The following results were obtained.

RELATIVE RESISTANCE OF FAMILIES TO TUBERCULOSIS

In the last column of table 1 are given the days of survival of each of the rabbits of the 6 families tested. The resistance as determined in this experiment is not, strictly speaking, the native resistance of these rabbits, for all had been injected with killed tubercle bacilli preliminary

TABLE 1

The relation in rabbit families between resistance to a standard intracutaneous inoculation of virulent tubercle bacilli and the spread of India ink in the skin and the development of allergic sensitivity and antibody production in response to preliminary treatment with heat-killed microorganisms

RABBIT NUMBER, AGE IN MONTHS AND SEX	INDIA INK SPREAD IN SQ. MM.		ALLERGIC SENSITIVITY BEFORE INFECTION		TITRE OF SERUM AGGLUTININS BEFORE INFECTION		SURVIVAL IN DAYS
	Before sensitization	On 65th day of infection	Rapidity of development of maximum sensitivity in days	Maximum tuberculin reaction in cmm. of inflammation	On eighth day after beginning of treatment	On fifty-fifth day after beginning of treatment	
A5=5, 14.2 ♂	208	379	47	176	16+	64+	791
A5=9, 11.0 ♂	256	506	52	120	16+	16+	189
A5=10, 11.0 ♂	441	484	37	266	64+	64+	182
A5=18, 9.0 ♂	550	361	37	1596	16+	64+	572
A5=19, 9.0 ♂	216	252	52	666	16++	64+	688
A5=20, 9.0 ♂	88	361	31	3693*	16+	64+	814†
Average	293	390	43	1086	24	56	539
F3-32, 23.0 ♂	575	756	52	76	0	16+	133
F3-33, 23.0 ♂	868	806	52	176	0	16+	132
F4-7, 13.0 ♂	1156	810	52	51	0	16+	149
F4-28, 9.5 ♀	1023	1364	47	157	0	16+	150
Average	905	934	51	115	0	16	141
C4S-3, 15.8 ♂	520	1360	52	171	16+	64+	88
C4S-7, 12.0 ♂	100	1665	52	325	64+	64+	155
C4R-3, 17.5 ♂	264	2310	23	270	16+	64+	80
C4R-9, 10.5 ♂	88	1170	23	600	16+	64+	89
C4R-10, 10.5 ♂	576	1344	52	101	64+	16+	194
Average	309	1570	40	293	35	54	121
B5-3, 14.2 ♂	353	354	52	124	16+	256+	302
B5-8, 10.0 ♂	448	288	37	220	64+	64++	222
B5-11, 9.0 ♀	960	1287	52	186	16+	64+	518
B5-14, 9.0 ♂	762	288	52	28	64+	64+	166
Average	631	539	48	139	40	112	302
H5=4, 7.5 ♀	1748	1360	52	270	4+	16+	347
H5=5, 7.5 ♂	1020	540	23	526	4+	16+	166
H5=6, 7.5 ♂	988	1394	52	23	16+	256+	252
H5=7, 7.5 ♂	552	432	31	753	0	64+	301
Average	1077	931	40	393	6	88	266

* Anaphylactic type of inflammation.

† Rabbit still living.

TABLE 1—*Concluded*

RABBIT NUMBER, AGE IN MONTHS AND SEX	INDIA INK SPREAD IN SQ. MM.		ALLERGIC SENSITIVITY BEFORE INFECTION		TITRE OF SERUM AGGLUTININS BEFORE INFECTION		SURVIVAL IN DAYS
	Before sensitization	On 65th day of infection	Rapidity of development of maximum sensitivity in days	Maximum tuberculin reaction in cmm. of inflammation	On eighth day after beginning of treatment	On fifty-fifth day after beginning of treatment	
D5=3, 9.0 ♀	960	1681	16	142	4+	16++	187
D5=4, 9.0 ♂	837	616	23	652	16+	16++	160
D6=R7, 9.5 ♀	990	936	23	1322	2+	16+	283
D6=R8, 9.2 ♂	702	564	9	1664	16+	64+	200
D6=R9, 9.2 ♀	1184	814	37	283	0	16+	189
Average.....	935	922	22	812	8	26	204

to their virulent infection. Nevertheless, since all the rabbits received identical preinfection treatment, the results obtained are a valid index of the relative resistance of these families under standard conditions and uniform dosage.

If the average survival time of each of the families in the experiment recorded in table 1 is listed in decreasing order and if at the same time the duration of the naturally acquired disease in the same families as described in the first paper of this series is listed in the same order, the following obtains:

AVERAGE LENGTH OF SURVIVAL AFTER STANDARD INOCULATION		AVERAGE DURATION IN MONTHS OF NATURALLY ACQUIRED DISEASE
Days	Months	
A* 539	18.0	A 9.3
B 302	10.0	B 6.9
H 266	8.8	H 6.5 (3rd and 4th generation)
D 204	6.8	D 6.1
F 141	4.7	C 5.3
C 121	4.0	F 4.9

* On July 22, 1941, one animal was still living.

It is clear that the relative resistance of these families was the same, irrespective of the method of infection, and was not fundamentally changed by immunization with killed tubercle bacilli. The A family was the most resistant; the F and C families were the most susceptible, and the

B, H and D rabbits were of intermediate resistance. This result supports the estimate of the duration of the disease in the naturally acquired infection as given in the first paper of the series. Furthermore it shows that the immunizing process increased the resistance of a family in proportion to its native resistance. The greater the native resistance the greater the acquired immunity. The acquired resistance was superimposed on the natural resistance of a given family.

The evidence for the relative resistance of the D family and for the fifth generation of the H family can now be presented. The average survival time of the H family was two hundred sixty-six days as contrasted with 539 for the A family on the one hand, and one hundred forty-one and one hundred twenty-one days for the F and C families respectively on the other. Statistical analysis according to the "Student" t curve of distribution shows that the differences in survival time between the H family on the one hand and the F and C families on the other are both significant, P being 99.1 and 99.4 respectively. Likewise the difference between the average survival time of the A and H families is of considerable statistical significance P being 95. Similar analysis of the average survival time of the D family, two hundred four days, as compared with that of the A, F and C families, places the D family in a position of intermediate resistance between them. The difference in survival time between the A and D families has a P value of 98, that between the D and C families a value of 98.8, and that between the D and F families a value of 97.9, all of which indicate that the observed differences are of considerable statistical significance.

It is clear, therefore, that the relative resistance of all these families as determined by natural respiratory contagion has been confirmed by statistically reliable differences in survival time following standard inoculation of uniform doses of tubercle bacilli parenterally administered.

THE SPREAD OF INDIA INK

In the second column of table 1 is recorded the spread of India ink in the skin of the members of the different families before any treatment was begun. This averaged 293 sq. mm. in the resistant rabbits of the A family. By contrast the value for the members of the F family was 905. The difference has a P value of 99.9. Thus a statistically significant correlation between resistance to tuberculosis and the relative spreading capacity of the skin in these two families is evident (figure 6 and 7). However, no such relationship existed in the remaining families. Thus

the rabbits of the C family limited the spread of the dye to practically the same average area as those of the A family; yet the C rabbits had consistently proved more susceptible than the A rabbits. Furthermore there is no constant relation between the spreading capacity of an individual rabbit in a given family and its resistance. Similar observations have been recorded in the first paper of this series.

Tuberculous infection had no significant effect on this skin property in 5 of these families. In family C, however, the effect was striking. In each of the 5 members of this family the spread of the dye following tuberculous infection was greatly increased. The difference is highly statistically significant, P being 100. It is conceivable that this greater spread was due to the general increased permeability of the tissues before death, as has been recorded by some observers (3). That this, however, is not the sole controlling factor is evident in rabbits C4S-7 and C4R-10, in which the test was performed ninety and one hundred thirty-five days respectively before death; yet the increment of the spread of the dye following infection was about 2 and 16 times that of their preinfection spread. The significance of this observation is not clear. It is possible however that just as the permeability of the skin to this particulate dye is increased in this family so may its tissues be more readily permeated by the bacilli as a result of an existing tuberculous infection. In the first two following columns the families detailed in table 1, are listed in the order of their average decreasing resistance, and in the parallel third column is given their corresponding average India ink spread on the sixty-fifth day of infection.

FAMILY	RESISTANCE AS INDICATED BY DAYS SURVIVED AFTER INFECTION	SPREAD OF INDIA INK IN SQ. MM.
A	539	390
B	302	539
H	266	931
D	204	922
F	141	934
C	121	1570

From this the impression might be gained that resistance is inversely related to the spreading capacity of the skin. Apparently the greater the spread the less the resistance to tuberculosis. Essentially similar results were obtained in the same 6 families in earlier inbred generations and were reported in a preliminary study (4). A closer examination, how-

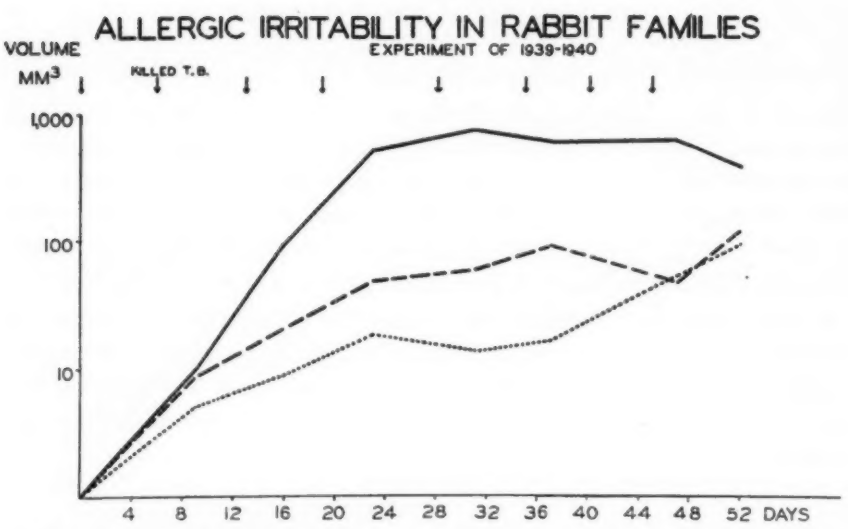
ever, shows that while this relationship holds in the families differing greatly in their resistance, as the A and B families on the one hand and the F and C families on the other, there is no corresponding difference in families that differ only moderately from each other. Furthermore this relationship does not hold in individuals of a given family. Thus in family B the female rabbit B5-11 spread the India ink more widely than the three remaining males of this family; yet it survived after infection for more than two hundred days longer than the others. Similar examples can be found in other families of this experiment. Therefore, suggestive though these observations are, one must hestitate to conclude that the spread of India ink in the skin of infected rabbits is a reliable index of their resistance to infection.

ALLERGIC SENSITIVITY

In columns 4 and 5 of table 1 are listed the data for the rate and intensity of development of maximum sensitivity to tuberculin in these rabbit families. It will be seen that the rabbits of the A, D and H families usually attained their maximum sensitivity more rapidly than those of the families F and B. The rabbits of the C family were very inconstant in this respect. Furthermore the same three families, A, D and H, attained a higher average maximum sensitivity than the families F, C and B. There was no constant relationship between the relative resistance of all the families and their innate capacity to develop allergic sensitivity in response to treatment with heat-killed tubercle bacilli from the standpoint both of the rate and intensity of the development of the phenomenon.

However, in three families, A, B and F, differing markedly in their resistance to tuberculosis, there was a corresponding graded difference in this capacity. This is illustrated in graph 1. The individual observations from which these composite graphs were constructed are given in table 2. It is clear from these that the most resistant A family developed allergic sensitivity fastest, the most susceptible F family the slowest and the B family, of intermediate resistance, developed allergic sensitivity at an intermediate rate. The degree of sensitivity developed in response to this nonviable antigen was similarly graded in these families. In the majority of the rabbits of the A family the tuberculin sensitivity attained was higher than in the B rabbits, while that of the B rabbits was usually higher than that of the F rabbits. Essentially similar differences were obtained in an earlier generation of these three families in a

GRAPH 1



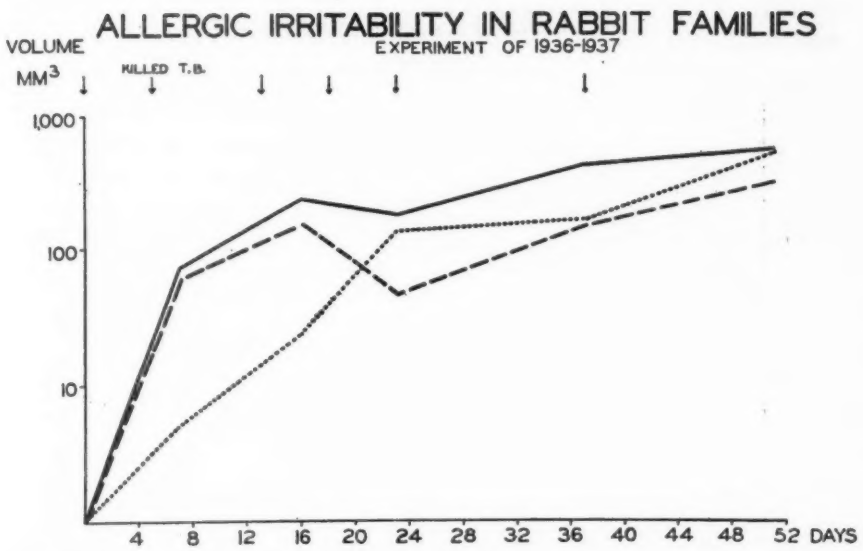
GRAPH 1. A family, resistant, ——; B family, intermediate, -----; F family, susceptible, ...

TABLE 2

Volume of inflammation of nodule in c.mm. two days after successive injections of tuberculin into the skin of rabbits of families A, B and F, during the course of treatment with heat-killed tubercle bacilli

RABBIT NUMBER THIRD, FOURTH AND FIFTH GENERATIONS	DAYS AFTER BEGINNING OF SENSITIZATION						
	9	16	23	31	37	47	52
A5=5	10	26	36	81	24	176	75
A5=9	9	14	31	46	76	95	120
A5=10	0	41	231	51	266	226	179
A5=18	31	326	761	633	1596	996	456
A5=19	0	18	328	235	57	300	666
A5=20	9	150	1728	3693	1260	2100	912
Average.....	10	96	519	789	629	649	401
B5-3	0	22	76	81	112	76	124
B5-8	0	10	76	97	220	47	151
B5-11	27	26	42	42	33	54	186
B5-14	9	28	5	20	6	21	28
Average.....	9	21	50	60	93	49	122
F3-32	0	3	9	7	0	8	76
F3-33	9	2	50	7	22	6	176
F4-7	5	22	9	18	31	42	51
F4-28	9	11	7	22	16	157	76
Average.....	5	9	19	14	17	53	95

GRAPH 2



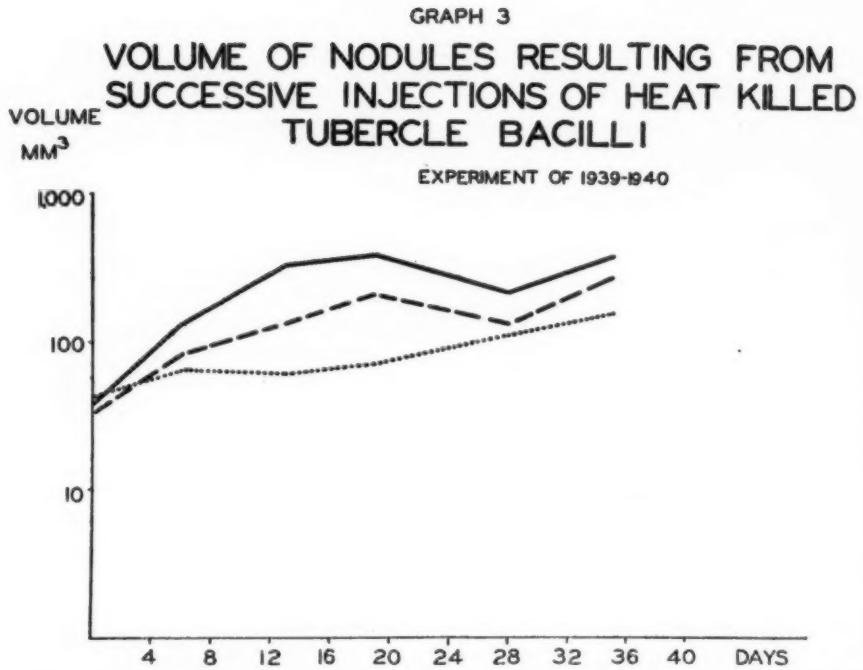
GRAPH 2. A family, resistant, ——; B family, intermediate, ——; F family, susceptible, ...

TABLE 3

Volume of inflammation in c.mm. two days after successive injections of tuberculin into the skin of rabbits of families A, B and F, during the course of treatment with heat-killed tubercle bacilli

RABBIT NUMBER, THIRD AND FOURTH GENERATIONS	DAYS AFTER BEGINNING OF SENSITIZATION				
	7	16	23	37	51
A3-6	21	72	9	339	812
A4-11	47	60	33	1015	638
A4-14	18	166	420	405	170
A4-17	108	675	281	68	720
Average.....	73	243	186	457	585
B4-14	167	320	47	381	423
B4-22	17	—	88	81	304
B4-23	3	0	14	16	262
Average.....	62	160	49	159	329
F3-15	10	0	207	252	331
F3-16	0	0	83	65	588
F3-18	9	97	276	184	338
F3-19	0	3	0	189	1040
Average.....	5	25	142	172	574

GRAPH 3



GRAPH 3. A family, resistant, ——; B family, intermediate, -----; F family, susceptible, ...

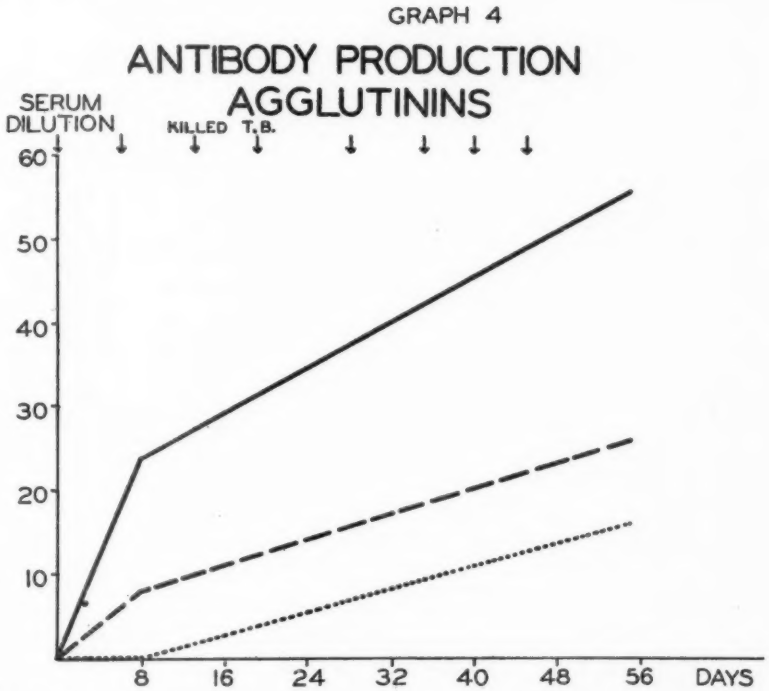
TABLE 4

Volume of inflammation in c.mm. two days after successive intracutaneous iniections of heat-killed tubercle bacilli in rabbits of families A, B and F

RABBIT NUMBER, THIRD, FOURTH AND FIFTH GENERATIONS	NUMBER OF NODULE AND DAYS ELAPSING AFTER BEGINNING OF TREATMENT					
	I, 0	II, 6	III, 13	IV, 19	V, 28	VI, 35
A5-5	28	48	78	138	192	86
A5-9	63	115	528	343	154	343
A5-10	28	163	504	356	403	560
A5-18	36	252	384	832	277	1155
A5-19	25	73	324	637	200	126
A5-20	45	144	187	40	80	36
Average.....	37	132	334	391	218	384
B5-3	48	78	172	189	316	144
B5-8	25	124	193	144	97	457
B5-11	21	86	62	183	80	368
B5-14	39	44	114	327	40	144
Average.....	33	83	135	211	133	278
F3-32	36	—	48	16	33	48
F3-33	48	44	27	54	79	81
F4-7	66	67	91	83	152	204
F4-28	24	84	78	134	192	300
Average.....	43	65	61	72	114	158

previous study (4). The detailed observations on these rabbits are given in table 3 and the composite averages are illustrated in graph 2.

Confirmatory evidence for the difference in rate and intensity of development of allergic sensitivity in these 3 families is given by the volume of the nodules resulting in these three families from successive intracutaneous injections of 1.0 mg. of heat-killed tubercle bacilli. This is illustrated in graph 3. The detailed observations from which these



GRAPH 4. A family, resistant, ——; D family, intermediate, ——; F family, susceptible, ...

composite graphs were constructed are given in table 4. It is evident from these that there is a graded relationship between the resistance to tuberculosis in these 3 families and the rate and intensity of development of allergic inflammation to heat-killed tubercle bacilli. Both the rate and intensity of inflammation were greatest with the most resistant family, least with the most susceptible and intermediate in the family of intermediate resistance.

TABLE 5

The relation in rabbit families between resistance to a standard intracutaneous inoculation of virulent tubercule baccilli and

(1) the progression and healing of the lesion at the portal of entry
(2) the degree of involvement of the draining axillary nodes
(3) the product of the number and extent of metastatic lesions
(4) the gross and microscopic character of the disease
(5) the quantity of tubercle bacilli in the lesions
following preliminary treatment with heal-killed microorganisms

RABBIT NUMBER	DAYS AFTER INFECTION WHEN LESION ATTAINED ITS MAXIMUM	DAYS AFTER INFECTION WHEN MAXIMUM HEALING WAS ATTAINED	MAXIMUM VOLUME OF LESION BEFORE HEALING BEGAN, IN C.MM.	PER CENT OF MAXIMUM HEALING OF LESION	VOLUME OF LESION AT DEATH IN C.MM.	VOLUME OF TBC. IN DRAINING AXILLARY NODE IN CC.	PRODUCT OF NUMBER AND EXTENT OF METASTATIC LESIONS	GROSS CHARACTER OF DISEASE	MICROSCOPIC CHARACTER OF LESION AT PORTAL OF ENTRY	TUBERCLE BACILLI IN LESION	SURVIVAL IN DAYS
A5-5	21	65	200	100	0	0	5	Healing, residual tbc.	Healed		791*
A5-9	29	86	230	99	sinus	0.7s	15	Chronic generalized tbc.	Epithelioid cells in sinus wall	Rare	189
A5-10	21	72	204	97	7	5.2s	9	Ulcerative pulmonary phthisis; spontaneous pneumothorax	Shallow ulcer with base of tuberculous granulation tissue containing "hard" tubercles	Occasional	182*
A5-18	36	572	627	94	40	0	17	Ulcerative pulmonary phthisis and epicarditis	Localized, ulcerated, softening, caseous lesion flanked by epithelioid cells and dense fibrous capsule; bacilli few in most cells, but swarming in some	Usually few; occasionally numerous	572
A5-19	15	93	307	100	38	0	12	Chronic fibrous tbc. with softening	Same as A5-18, but bacilli are absent from cells	Few	688
A5-20	21	65	202	100							814‡
Average...	24	79**	295	98							539

F3-32	57	93	1432	34	1872	5.7c	25	Rapidly progressive generalized tbc.	Entirely caseous, teeming with bacilli even in peripheral living cells; extension by perivascular lymphatics with swarms of bacilli	Massive	133
F3-33	72	93	1366	33	1474	3.0c	19	Rapidly progressive generalized tbc.	Essentially the same as F3-32	Massive	132
F4-7	65	86	950	31	816	2.3c	22	Rapidly progressive generalized tbc.	Essentially the same as F3-32	Massive	149
F4-28	50	79	490	78	327	2.4c	15	Rapidly progressive generalized tbc.	Essentially the same as F3-32	Massive	150
Average...	61	88	1059	44	1122	3.3c	20				141
C4S-3	65	79	1600	48	768	2.5c	15	Rapidly progressive generalized tbc.	Fibrous tbc. with discrete caseous foci extending by perivascular lymphatics and peripheral epithelioid cells with swarms of bacilli	Numerous	88
C4S-7	57	155	1783	43	1020	1.3c	25	Rapidly progressive generalized tbc.	Completely caseous; bacilli numerous at periphery; no softening	Numerous	155
C4R-3	57	79	749	50	380	0.5c	8	Rapidly progressive generalized tbc.	Encapsulated, massively caseous lesion; peripheral epithelioid cells swarm with bacilli. These spread between collagen bundles leading to their necrosis *en masse*	Innumerable	80
C4R-9	44	89	646	95	36	0	3	Slight generalized tbc.	Numerous bacilli in caseous base of ulcer. Tuberculous, fibrous granulation tissue with frequent bacilli in epithelioid cells. Lesion well encapsulated; some perivascular lymphatic extension	Numerous in caseous tissue; few in epithelioid cells	89*
C4R-10	44	72	535	58	2764	0.9c	17	Chronic generalized tbc.	Massively caseous; moderate numbers of bacilli; they are numerous in foci of beginning softening; extension by perivascular lymphatics and by peripheral cells, both with numerous tubercle bacilli	Numerous	194
Average...	53	79**	1063	59	994	1.0c	14				121

TABLE 5—*Concluded*

RABBIT NUMBER	DAYS AFTER INFECTION WHEN LESION ATTAINED ITS MAXIMUM	DAYS AFTER INFECTION WHEN MAXIMUM HEALING WAS ATTAINED	MAXIMUM VOLUME OF LESION BEFORE HEALING BEGAN, IN C.MM.	PER CENT OF MAXIMUM HEALING OF LESION	VOLUME OF LESION AT DEATH IN C.MM.	VOLUME OF TBC. IN DRAINING AXILLARY NODE IN CC.	PRODUCT OF NUMBER AND EXTENT OF METASTATIC LESIONS	GROSS CHARACTER OF DISEASE	MICROSCOPIC CHARACTER OF LESION AT PORTAL OF ENTRY	TUBERCLE BACILLI IN LESION	SURVIVAL IN DAYS
B5-3	36	128	396	98	140	4.8s	18	Chronic generalized tbc. with pulmonary cavities	Encapsulated, softened lesion extending by contiguity and perivascular lymphatics	Usually moderate; occasionally numerous	302
B5-8	36	128	134	100	0	0	15	Chronic generalized tbc.	Completely healed		222
B5-11	29	65	149	100	0	14.0s	14	Chronic generalized tbc. with pulmonary cavities	Completely healed		518
B5-14	36	86	369	96	69	1.0s	16	Chronic generalized tbc.	Essentially the same as B5-3; bacilli are numerous in softened areas, in peripheral cells and in perivascular lymphatics	Numerous	166
Average...	34	102	262	98	52	5s	16				302
H5=4	36	108	428	100	39	0.2†	8	Slight chronic generalized tbc.	Completely encapsulated caseous lesion with foci of calcification and softening, the latter only containing tubercle bacilli	Few, except in softened foci	347*
H5=5	50	65	544	26	576	0	14	Chronic generalized tbc.	Poorly delimited, partially encapsulated lesion extending by perivascular lymphatics and by invasion of distant collagen bundle spaces with bacilli, free or in cells	Massive	166
H5=6	29	79	604	100	0	1.0s	9	Chronic generalized tbc.	Completely healed except for failure of epithelium to grow over denuded collagen	0	252
H5=7	36	79	221	100	11	0	13	Chronic generalized tbc.	Completely caseous, softening lesion with few bacilli extending by perivascular lymphatics and by invasion of collagen bundle spaces with cellular debris	Few	301
Average...	38	83	449	81	156	0.3	11				266

Average...	38	83	449	81	156	0.3	11				266

D5-3	36	93	216	60	220	9s	9	Chronic generalized tbc.	Caseous lesion with areas of softening; bacilli numerous in latter; these are few in granulation tissue and in the perivascular lymphatics	Usually few; numerous in softened foci	187*
D5-4	44	100	368	91	23	2.9s	8	Chronic generalized tbc.	Sharply delimited, encapsulated caseous lesion; bacilli few everywhere; slight lymphatic extension	Few	160
D6-R7	15	100	273	97	988	0.7s	24	Extensive, slowly progressive generalized tbc.	Completely caseous, partially encapsulated lesion with foci of softening; bacilli numerous everywhere, especially in intact peripheral cells	Numerous	283
D6-R8	50	100	573	95	30	0.4s	19	Extensive, slowly progressive generalized tbc.	Small, sharply delimited, completely encapsulated caseous lesion with focal liquefaction; bacilli few everywhere; occasionally numerous in lymphatics	Usually few; rarely numerous	200
D6-R9	15	93	342	99	10	0.2s	7	Slight chronic generalized tbc.	Essentially the same as in D6-R8	Usually few; rarely numerous	189*
Average...	32	97	354	88	254	2.6s	13				204

* Complicated by intercurrent disease.
** Median instead of average.
† Calcification.
s=Softened.
c=Caseous.
§ Rabbit still living.

ANTIBODY PRODUCTION

In columns 6 and 7 of table 1 are given the titre of agglutinins against the tubercle bacillus which were demonstrated in the serum of these rabbits on the eighth and the fifty-fifth day after beinning of treatment, respectively. As was said above for the development of allergic sensitivity, there was no unvarying relationship between the rate and intensity of antibody production and resistance to tuberculosis in all these families. Nor was there an unvarying relationship between the development of allergic sensitivity and the production of antibodies. However, three families, A, D and F, differing greatly in resistance to tuberculosis, also differed correspondingly in their capacity for antibody production, both from the standpoint of the rate and intensity of the phenomenon. This is illustrated in graph 4. The most resistant A family produced antibodies most rapidly and to the highest titre; the most susceptible F family produced agglutinins most slowly and to the lowest degree. The D family, of intermediate resistance, produced antibodies at an intermediate rate and to an intermediate degree.

In table 5 the relative resistance of these families is analyzed from the standpoint of the rate and intensity of development of local immunity, the degree of generalization, and the gross and microscopic characters of the disease.

DEVELOPMENT OF LOCAL IMMUNITY

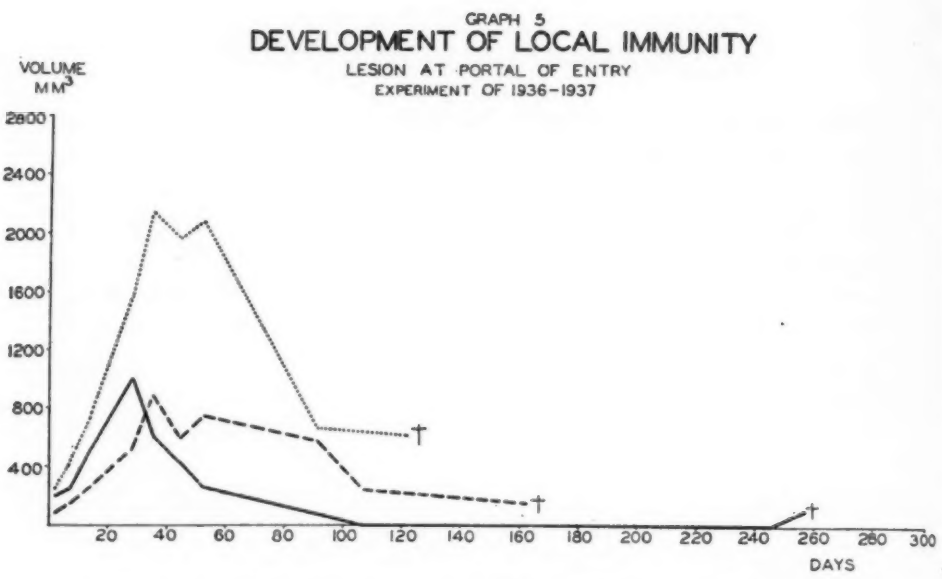
In the second column of this table are listed the number of days rerequired by the members of all the families for the local lesion to attain its maximum development before healing set in.

It will be seen that in the A family healing began after an average of twenty-four days; in the B, D and H families it began after thirty-four, thirty-two and thirty-eight days respectively. In the C and F families, however, healing did not begin before an average of fifty-three and sixty-one days, respectively, had elapsed. In other words, appreciable local immunity developed most rapidly in the most resistant A family, most slowly in the two families of lowest resistance and at an intermediate rate in the three families of intermediate resistance.

The degree of healing in each of these rabbits is recorded in per cent in column 5. This gives the maximum healing that took place at any time in the course of the disease It does not represent the lesion at death, which is given in column 6, for, in the susceptible families in par-

ticular, abortive healing occurred only to be followed by marked exacerbation later. This percentage healing was determined as follows. The early maximum volume of the lesion was considered 100 per cent. The percentage healing as recorded represents the difference between the volume at the time of maximum healing and the volume at the period of its early maximum development. It may be seen that this healing was nearly complete and equally so in the A and B families, averaging 98 per cent in each. However, maximum healing was usually achieved earlier in the A than in the B family. In the members of the H and D families healing was usually equally pronounced; occasionally, however, a member of these families failed considerably in this respect. The average healing in these families was 81 and 88 per cent respectively. Healing was least in evidence in the two most susceptible families. In the C family only one out of the 5 rabbits healed his lesion conspicuously; all the others failed quite as conspicuously, so that the average healing in this family was only 59 per cent. The rabbits of the F family uniformly failed to heal their lesion to any marked extent and averaged only 44 per cent. It is clear, therefore, that not only was the rate at which local immunity developed related to the resistance of these rabbit families but the intensity and effectiveness of the process was equally definitely correlated with their relative resistance. Healing was most complete and most rapidly achieved in the most resistant A family. It was least pronounced in the susceptible families, F and C, and was of an intermediate degree in the families of intermediate resistance.

Again we find that the F family was most uniform in its response at the portal of entry of the bacillus. The greatest majority of the members of the C family reacted in a similarly uniform manner. Occasionally, however, a rabbit in this family was encountered which developed quite a pronounced local immunity. The families of intermediate resistance B, D and H also showed some heterogeneity in their response. The A family, on the other hand, was relatively homogeneous both in the rate and effectiveness of its development of local immunity. It will be remembered from the first paper of this series that the A family was found to be uniformly highly resistant to tuberculosis acquired by natural respiratory contagion, the F family unvaryingly highly susceptible to this mode of infection and the C family, in the great majority of instances, equally susceptible. Occasionally however a member of this family showed considerable resistance. Finally families B, D and H were found to be mixed in their response to this infection and, correspondingly,



GRAPH 5. A family, resistant, ——; B family, intermediate, -----; C family, susceptible, ...

TABLE 6

The development of local immunity in families of varying resistance

Volume in c.mm. of the lesion at the portal of entry after a standard intracutaneous inoculation of virulent living tubercle bacilli following preliminary treatment with heat-killed microörganisms

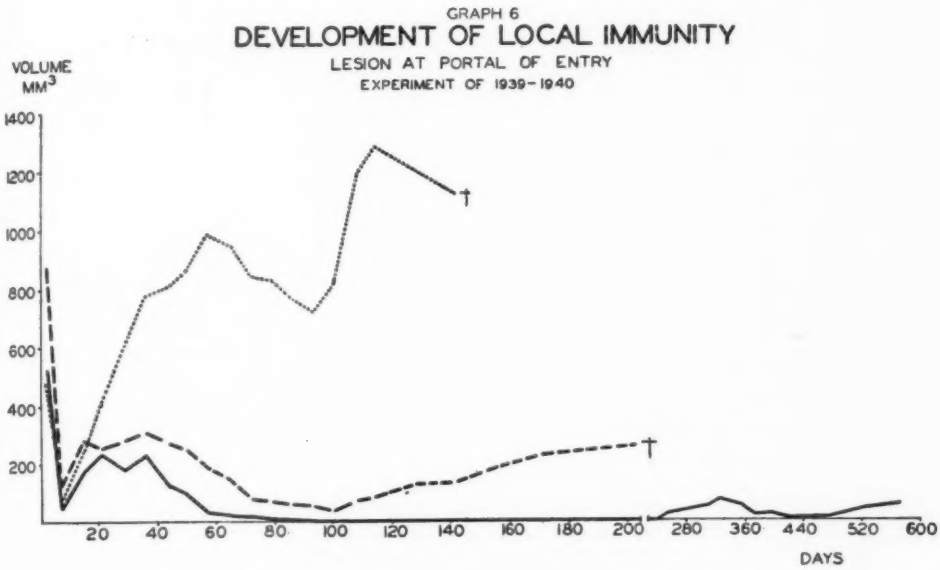
RABBIT NUMBER	DAYS AFTER INFECTION									VOLUME AT DEATH	SURVIVAL IN DAYS
	2	7	14	28	35	44	52	91	107		
A3-6	266	459	880	1315	945	543	388	33	0	0	157*
A4-11	135	373	420	1201	547	277	114	0	0	0	276
A4-14	79	57	234	491	331	252	237	264	441	108	172*
A4-17	312	80	544	1016	621	684	302	0	0	317	424
Average.....	198	242	519	1006	611	439	260	74	0**	106	257
B4-14	100	140	221	581	970	554	756	400	230	56	158*
B4-22	44	187	309	722	1110	798	977	997	226	218	155
B4-23	72	105	256	228	604	436	538	375	315	374	174
Average.....	72	144	262	510	894	596	757	591	251	162	162
C3-12	440	724	1056	1481	1674	1242	680	36		0	182*
C3-13	100	226	475	1200	1820	1724	2240	561		561	91
C3-14	132	145	448	1419	1764	2400	2092	1419	1614	646	122
C3-16	308	611	1012	2163	3298	2553	3321			1276	88
Average.....	245	426	748	1566	2139	1979	2083	672		621	121

* Tuberculosis plus intercurrent disease.

** Median not average.

the development of local immunity was likewise mixed in its nature. Thus a positive correlation is established between the resistance of all the families and the rapidity and intensity of their development of local immunity as judged by the progression and healing at the portal of entry of these uniformly treated rabbits.

In table 6 are given the individual observations of the volume of the lesion at the portal of entry at different intervals following the intracutaneous injection of 0.2 mg. Ravenel strain tubercle bacilli in members of either the third or fourth generations of the families A, B and C re-



GRAPH 6. A family, resistant, ——; D family, intermediate, – – –; F family, susceptible, ...

spectively, of high, intermediate and low resistance to tuberculosis. Data on the relative resistance in those families were reported in summary in a preliminary communication (4). Graph 5 gives the composite curves of the fate of the lesion in these families.

In table 7 and graph 6 are presented the corresponding observations of the present study on the same highly resistant A family, but of the fifth inbred generation. The behavior of these rabbits is here compared to that of two other families, the F family of great susceptibility and the D family of intermediate resistance.

TABLE 7

The development of local immunity in families of varying resistance

Volume in c.mm. of the lesion at the portal of entry after a standard intracutaneous inoculation of virulent living tubercle bacilli following preliminary treatment with heat-killed microörganisms

RABBIT NUMBER	DAYS AFTER INFECTION																					VOLUME AT DEATH
	2	7	15	21	29	36	44	50	57	65	72	79	86	93	100	108	156	207	312	398	572	
A5=5	372	48	163	200	140	157	72	39	5	0	0	0	0	0	0	0	0	0	0	0	0	0
A5=9	310	44	127	225	230	192	132	100	56	26	36	16	0	0	scab	2	3	†				0
A5=10	522	66	172	204	118	115	46	44	33	19	7	6	7	12	14	7	10	†				7
A5=18	453	40	172	300	280	627	297	272	540	486	464	453	225	312	235	285	238	273	166	46	40†	40
A5=19	1040	86	307	270	208	204	151	81	40	30	43	19	10	0	0	0	0	0	66	63	183	38
A5=20	475	7	110	202	115	93	56	33	8	0	0	0	0	0	0	0	0	0	44	0	0	
Average	529	48	175	233	182	231	126	95	36*	22*	21*	11*	3*	0*	0*	1*	1*	0*	55	27	56	
D5=3	207	25	127	214	184	216	153	97	149	243	112	115	118	86	108	154	608	†				220
D5=4	540	126	300	266	264	340	368	201	210	144	88	68	70	88	33	88	52	†				23
D6=R7	2444	260	273	172	226	144	166	168	180	54	68	60	41	27	9	46	189	1092	†			988
D6=R8	725	85	342	266	434	529	460	573	319	192	112	96	67	56	28	60	60	†				30
D6=R9	476	109	342	326	307	299	234	210	98	85	18	18	9	3	4	17	10	†				10
Average	878	121	277	249	283	305	276	250	191	143	79	71	61	52	36	73	184	1092				
F3-32	736	127	285	478	883	900	832	1142	1432	1364	1140	1152	1064	952	986	1508	†					1872
F3-33	392	92	324	547	760	1108	1113	1159	1294	1214	1366	1288	1188	920	950	1531	†					1474
F4-7	756	60	353	522	567	798	950	673	883	950	699	777	652	876	1044	1320	†					816
F4-28	96	14	32	96	225	309	331	490	332	261	183	108	153	132	259	403	†	.				327
Average	495	73	248	411	608	779	806	866	985	947	847	831	764	720	810	1190						

* Median not mean.

† Rabbit died.

Examination of these data leaves no doubt that the A family in both experiments developed a high degree of local immunity at the most rapid rate. The C family, of lowest resistance, in one experiment, and the F family, of corresponding resistance, in the other experiment, both showed the slowest development and least effective local immunity. The B family, of intermediate resistance, in one experiment and the D family, of the same relative resistance, in the other experiment, showed an intermediate rate and degree of development of local immunity (figures 3, 4 and 5). It is noteworthy that even without preliminary immunization essentially the same differences appeared in the local lesions of these families, although healing was never complete even in the A family under these conditions. In figures 1 and 2 these differences are illustrated in a member of the family A and in one of the second inbred, susceptible generation of the H family.

Two observations recorded in these tables and graphs require some discussion. It will be noted in the first experiment that the volume of the lesion was usually smaller 2 days after inoculation than on the seventh day of the infection. In the second experiment, however, the lesion on the second day of infection was invariably larger than that on the seventh day. In other words the rabbits of the second experiment showed a mild Koch phenomenon on injection with living bacilli, following their preliminary treatment with killed microörganisms. The intensity of this early inflammation represents the degree of hypersensitivity to living tubercle bacilli that these rabbits acquired at the time of their virulent infection. While these two experiments were done at widely separated intervals, yet the same rabbit families were used and the same dose and strain of living microörganisms were inoculated intracutaneously. There is no evidence that the virulence of the Ravenel strain changed during this interval, nor are there any observations indicating that the resistance of the rabbit families tested had altered during this time. The rabbits in the old experiment received fewer injections of heat-killed microorganisms prior to their infection with living bacilli than those of the present study. It is not surprising, therefore, to find that family A, of the present study developed a far higher allergic sensitivity to living bacilli (table 7 and graph 6) than the same family of the preceding study (table 6 and graph 5). Furthermore, families B and D of the old experiment, which are not given in detail here, were also much less sensitive to virulent tubercle bacilli than the same families in the present experiment. It is interesting to find, therefore, in this connection that the

† Rabbit died.

average survival of these families in the present experiment was conspicuously greater than that of the same families similarly infected in the preceding study. Furthermore, the survival of the C and F families in both studies did not differ appreciably nor did their sensitivity to living bacilli differ conspicuously in these two experiments. These results do not justify the conclusion that a greater allergic sensitivity is associated with a greater resistance to infection. They do not, however, speak against the implication that the capacity to become sensitized parallels the capacity to become immunized, especially since it was found in this study that the capacity of a family for development of a high allergic sensitivity in response to treatment with heat-killed tubercle bacilli is associated with a high resistance to tuberculosis. Finally, the immunity of the families of high and intermediate resistance could be increased by increasing the preliminary treatment with heat-killed tubercle-bacilli. This could not be accomplished in families of high natural susceptibility to the infection.

FIG. 1. (Upper left.) Local lesion of rabbit A3-9 on the fifty-fifth day following an intracutaneous inoculation of 0.2 mg. Ravenel strain of bovine type tubercle bacilli. Small ulcerated lesion without involvement of draining axillary nodes. The protocol of this rabbit may be found in paper II. Rabbit survived two hundred and ninety-one days.

FIG. 2. (Upper right.) Local lesion of rabbit H2-23 of the second, susceptible, inbred generation of the H family on the fifty-fifth day following the same inoculation as in rabbit A3-9 shown in figure 1. The local lesion is much larger and the draining axillary node is enlarged and tuberculous. Rabbit survived one hundred and thirty-three days.

FIG. 3. (Center left.) Local lesion of rabbit F3-32 on the one hundred and seventeenth day after intracutaneous infection of 0.2 mg. Ravenel strain bovine type bacilli following preliminary treatment with heat-killed tubercle bacilli. There is a large ulcerated lesion extending to the neighboring draining lymph nodes, which are massively tuberculous. Rabbit survived one hundred and thirty-three days.

FIG. 4. (Center right.) Local lesion of rabbit D5=4 on the sixtieth day following the identical treatment, as recorded for rabbit F3-32 in figure 3. Both the local lesion and the draining lymph nodes are definitely less involved than these structures in F3-32, figure 3. Rabbit survived one hundred and sixty days.

FIG. 5. (Lower left.) Local lesion of rabbit A5=5 on the sixtieth day following identical treatment, as recorded for rabbits F3-32 and D5=4, the lesions of which are shown in figures 3 and 4, respectively. The lesion is almost completely healed. The draining nodes are not affected. Rabbit survived seven hundred and ninety one days.

FIG. 6. (Lower center.) The spread of 0.5 cc. India ink in the skin of rabbit F3-28 on the fifteenth day after an intracutaneous injection.

FIG. 7. (Lower right.) The spread of India ink in the skin of A4=16 on the fifteenth day after the intracutaneous injection of the same amount of India ink as in rabbit F3-28 (shown in figure 6).

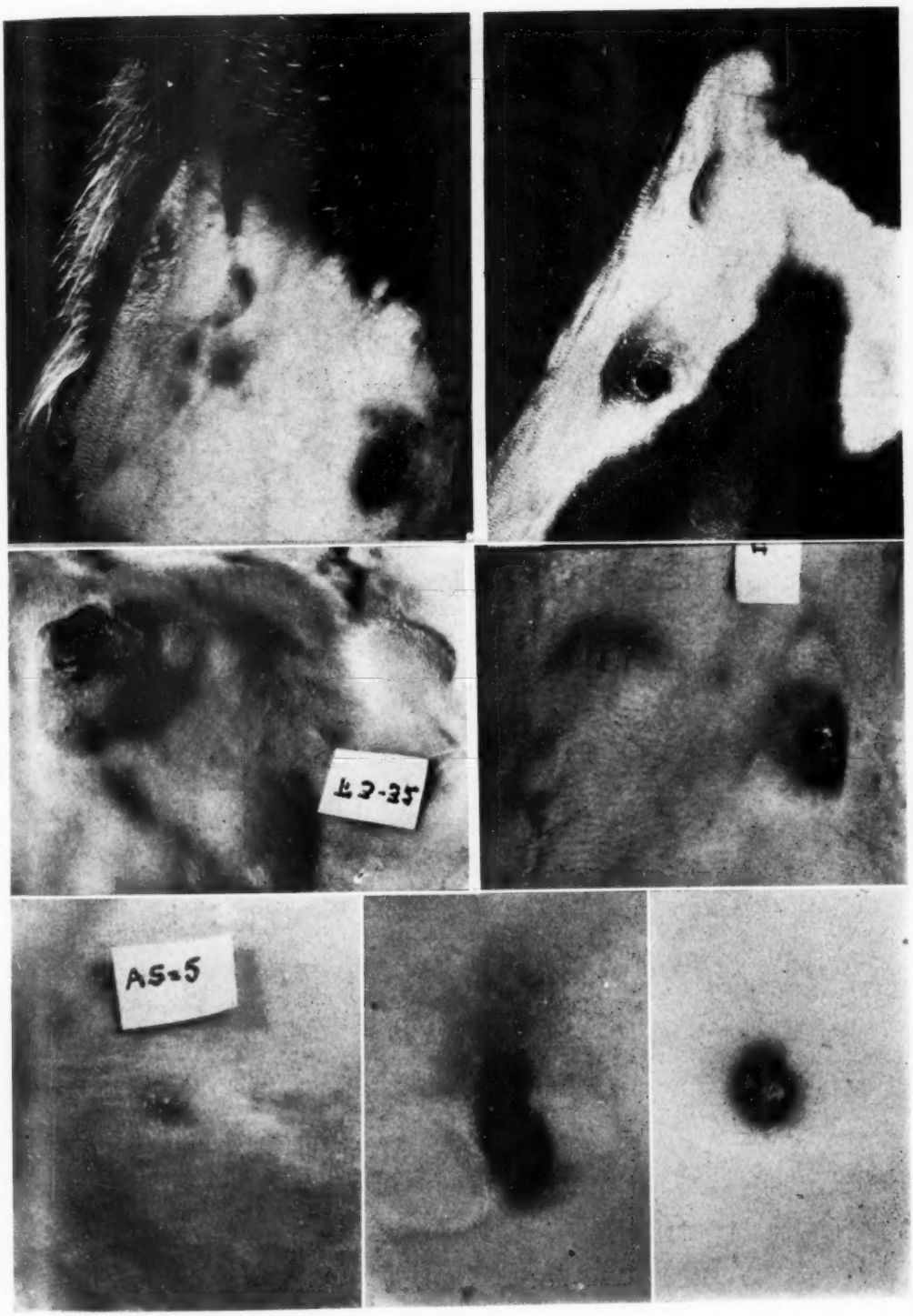


FIG. 1–7

Another observation made in the present study (table 7 and graph 6) seems worthy of notice. The progress of local immunity as indicated by the healing of the lesion was rapid at first and then waned so that even in the highly resistant A family an exacerbation of the lesion occurred. This exacerbation occurred early in the susceptible family, following which the lesion attained an even greater development than that prior to the onset of early healing. It set in later in the family of intermediate resistance and did not quite reach the degree of development of the lesion prior to the onset of healing. In the most resistant A family this exacerbation appeared very late in the course of the disease and resulted only in a slight reinflammation of the practically healed lesions of some rabbits. The exact significance of this phenomenon is not clear; but its importance cannot be overlooked, for this waning of immunity is apparently responsible for the ultimate death of even the highly resistant members of the A family, after the immunity had kept in check the progress of the disease for such a long time. There are some observations suggesting that the cells which had inhibited the growth of the bacilli within their cytoplasm up to the onset of the exacerbation now permitted them to grow again in their substance. This is evidenced by the presence of large numbers of bacilli within intact mononuclears even in members of the resistant family A. Similar observations were reported in 1928 (5). Rabbits infected with the human type of tubercle bacillus that had almost completely destroyed the microörganisms in the spleen at the end of four months, as demonstrated by culture, showed a secondary increase two months later.

DISSEMINATION OF THE DISEASE FROM THE PORTAL OF ENTRY

Returning to table 5, we find that the relative resistance of these rabbit families may be correlated with the degree of involvement of the lymph nodes draining their portal of entry. It will be noted in column 7 that in 4 out of the 6 rabbits of the A family these nodes were free from gross tuberculosis at death. Similarly in two rabbits of the H family and in one rabbit each of the B and C families these nodes were free from tuberculosis at this time. It is important to emphasize, however, that each one of these rabbits showed unequivocal enlargement and induration of these nodes on palpation in the first two months following their inoculation. It seems fairly certain that this enlargement was caused by an ephemeral tuberculosis, which healed 2 months later, at which time these nodes could no longer be felt. The two rabbits of the A family, A5-9

and A5=10, in which the draining nodes did not heal, died earliest of their tuberculosis. The remaining four rabbits of this family far outlived these two rabbits. It may be of some significance in this relation to state that the latter 4 rabbits were litter mates, descending from the same parents (A4=15 and A4=16 (see chart 1). The former two rabbits, however, were descended from the same father, A4=16, but from a different mother, A4-18.

Thus in 4 out of 6 rabbits of the A family the nodes draining the site of inoculation were free from tuberculosis at death, just as the tracheobronchial nodes draining the primary pulmonary focus also were free from gross disease in this family in the infection acquired by natural respiratory contagion. In the naturally acquired disease this healing of the intrathoracic tracheobronchial nodes could not be observed; it is likely, however, that, had this been possible, similar observation might have been made.

Despite the healing of both the skin lesion at the portal of entry and of the immediate draining nodes in the majority of the members of the highly resistant A family, nevertheless the disease progressed in the distant internal organs, leading to the ultimate death of these rabbits. These are obviously instances of the fact demonstrated in another study (6) that on local infection, immunity develops most rapidly at the portal of entry, and the further removed the metastatic lesion is from that point the less marked is this immunity at a given time. On this basis we can understand the healing of the lesion at the portal of entry and even in the immediate draining nodes while at the same time the disease slowly progressed in the lungs and other distant foci of localization, leading to the death of the individual.

In the remaining rabbits of this study the draining nodes were more or less involved. In the susceptible families, F and C, these nodes were caseous at death, but in the families of intermediate resistance, B, D and H, they had undergone softening before death. In many instances this difference in the pathological state of the draining nodes may be attributed to the shorter duration of the disease in the susceptible animals. In other instances however this cannot be the cause. For example in rabbits C4S-7 and C4R-10 of the susceptible family C, which survived one hundred and fifty-five and one hundred and ninety-four days respectively, no softening had overtaken the draining nodes at death. In rabbits D5=4 and D5=3 of intermediate resistance, which died in approximately the same time, one hundred and sixty and one hundred and eighty-

seven days respectively, the nodes had already undergone softening when they died. This suggests that time alone does not account for the observed difference. It is more likely that just as the development of allergic sensitivity, antibody production and local immunity are more rapid in the more resistant animal so the mechanism responsible for the development of softening also comes into play earlier in the more resistant animal.

The extent of dissemination of the disease to the rest of the body is given in numerical form in column 8 of table 5. It will be noted that the number and extent of the metastatic lesions tends to be greater in the more susceptible families. However, this is not invariably the case. The great differences in survival of the different families may account for the irregularities observed in this relation.

GROSS CHARACTER OF THE DISEASE

The general character of the disease as developed by the different families bore a definite relationship to their resistance. In the most resistant A family ulcerative pulmonary phthisis was the dominating picture of the disease. In the families of intermediate resistance, B, D and H, the disease had the characteristics of a slowly progressive generalized tuberculosis. Occasionally a rabbit of the B family developed pulmonary cavities. In the uniformly highly susceptible F family the disease had the uniform earmarks of a rapidly progressive generalized tuberculosis. The majority of the rabbits of the susceptible C family developed a disease of similar nature. Two of the rabbits of this family, however, died from a slowly progressive generalized infection. Again the C family showed itself to be of mixed composition.

MICROSCOPIC CHARACTER OF THE DISEASE

A searching light on the mechanism of the varying resistance to tuberculosis in these families was afforded by microscopic study of the lesion at the portal of entry of the virulent infection and also by an examination of the nodule resulting from the third injection of killed tubercle bacilli prior to their virulent infection.

The second of these items is simpler and may be readily stated. The nodule resulting from the third successive intracutaneous injection of heat-killed tubercle bacilli removed on the second day after injection differed in these families in the following respects. In the susceptible families the disruption of the collagen bundles by the inflammatory

exudate was extensive. Large masses of exudative cells were found in which collagen fibres were not seen. In the highly resistant A family many collagen bundles remained in the form of a coarse network, in the meshes of which the exudate accumulated. Bacilli were numerous, and in large clumps in the exudate of the susceptible animals. In the exudate of the resistant animals the bacilli were clearly fewer and more dispersed among the phagocytic exudative cells. Mononuclears were numerous at the periphery of the nodule and their cytoplasm was abundant in the rabbits of the resistant family. New blood capillaries had also formed. The mononuclears were distinctly fewer and their cytoplasm much less abundant in the nodules formed by the susceptible families in response to the same irritant. Blood capillaries were not seen in these nodules. These observations require repetition for confirmation. Nevertheless they suggest that possibly the cohesiveness of the connective tissue in the highly resistant A family is greater than that in the highly susceptible rabbits. The phagocytosis of tubercle bacilli is possibly more active in the resistant than in the susceptible family. The mobilization and maturation of the mononuclears is apparently more pronounced in the resistant families. Likewise the growth response of the blood capillaries is more active in the resistant family.

Under the heading of microscopic character of the lesion at the portal of entry and in the following column in table 5 are given the essential histologic observations relative to each of these rabbits. It will be seen that in the A family there were either no active tuberculous changes at all or a sinus, a softened focus or ulcer was found, surrounded by tuberculous fibrous granulation tissue with "hard" epithelioid-cell tubercles containing but few bacilli (figure 8). Such lesions were sharply localized and encapsulated. Each had the essential structure of the slowly progressive primary focus seen in the lung of those rabbits that acquired their tuberculosis by natural respiratory contagion. The only difference between them is the anatomical structures of the tissues involved. The only mode of extension available in the skin was by haematogenous and lymphogenous channels. As was found in the rabbits with the naturally acquired disease of pulmonary origin, so, in a disease originating in the skin, this mode of extension played a minor rôle in the progress of the infection. However, in the lung the disease extended by canalicular spread; hence the progression of the disease in the lungs. In the skin this mode of extension was not available; hence the complete healing of the primary lesion in the majority of these rabbits. Ap-

parently the mononuclear of the A family rapidly acquired an increased capacity effectively to inhibit the growth of the bacilli.

By contrast the lesion at the portal of entry in the skin of the uniformly highly susceptible F family was completely caseous and teeming with bacilli. Even in the peripheral, still living mononuclears the bacilli were swarming (figure 11). Intact mature epithelioid cells were scanty. The lesion extended in all directions by invading distant portions through the perivascular lymphatics. These structures formed rings containing actual colonies of bacilli around blood vessels the lumen of which was frequently obliterated by the concentric multiplication of cells in the media (figure 9). There was little if any encapsulation of the lesions in the F family. It is clear that even after preliminary immunization with killed tubercle bacilli and after a disease of as much as one hundred and fifty days duration the mononuclears of the F family had not yet acquired sufficient capacity to interfere with the unhindered multiplication of the bacilli in their cytoplasm. The lymphatic pathways lay open for their invasion by cells laden with rapidly growing microorganisms. The lesion underwent caseation *en masse* and teemed with bacilli. Death took place before softening set in.

In the C family the disease was not quite so fulminating, fibrous tissue was formed and, at times, encapsulation was quite advanced. Caseation was not quite so massive. In one rabbit even minute foci of beginning softening were encountered. Still the peripheral epithelioid cells

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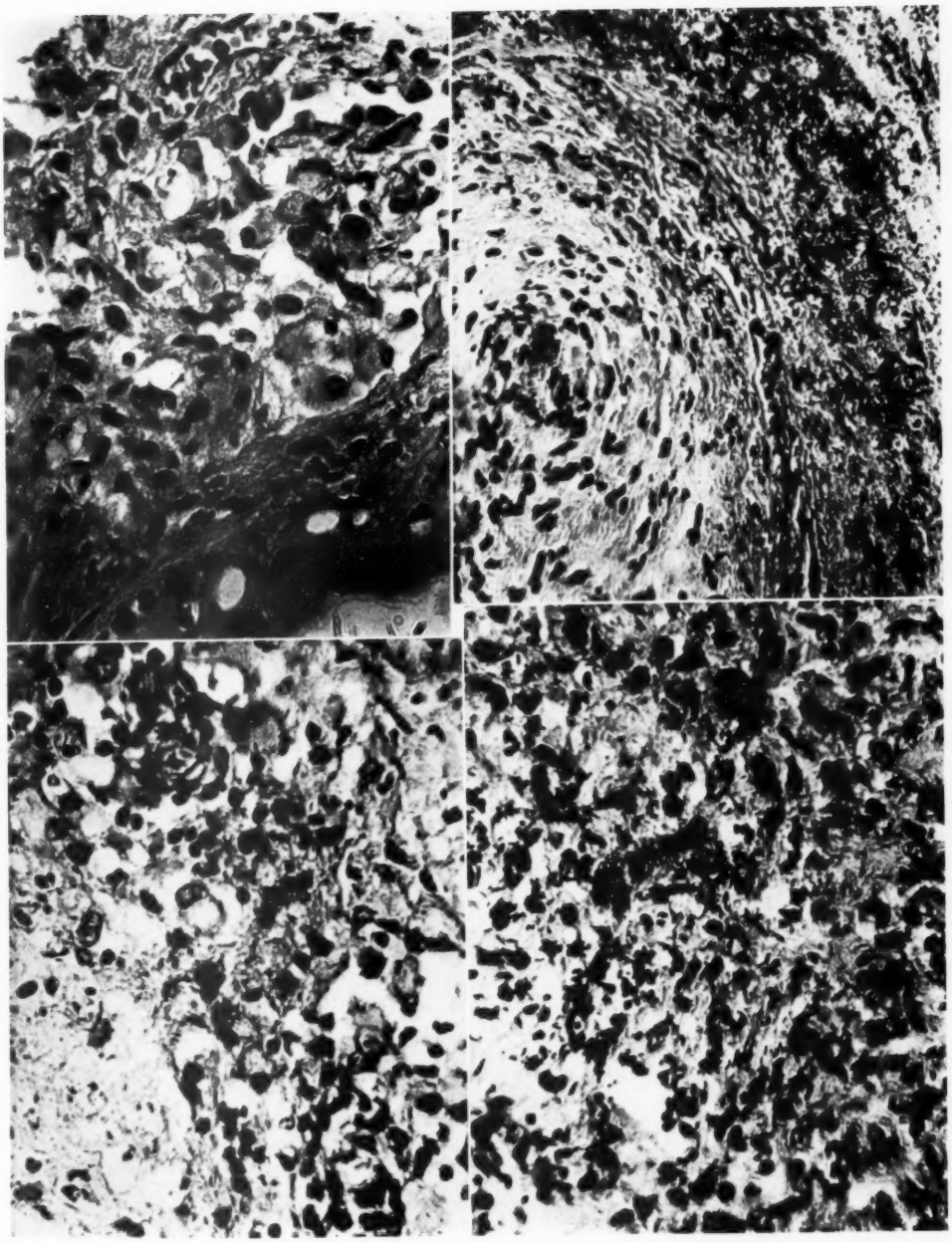
The photomicrographs presented in figures 8 to 11 inclusive were made from sections stained for tubercle bacilli with the Ziehl-Neelsen method and counter-stained with haematoxylin. The magnification in all these figures is about 500.

FIG. 8. (Upper left.) The wall of a small sinus remaining in the skin of rabbit A5-9, one hundred and eighty-nine days after an intracutaneous inoculation of 0.2 mg. Ravenel strain of bovine type bacilli following preliminary treatment with heat-killed tubercle bacilli. Mature epithelioid cells rarely contain tubercle bacilli. Only one microörganism can be seen within such an epithelioid cell, at about the center of the figure.

FIG. 9. (Upper right.) Local lesion of rabbit F4-7 on the one hundred and forty-ninth day following the identical treatment given rabbit A5-9, a lesion from which is shown in figure 8. A blood vessel is shown, the lumen of which is occluded by concentric multiplication of subendothelial cells. Surrounding this vessel there is a perivascular lymphatic swarming with tubercle bacilli.

FIG. 10. (Lower left.) Local lesion of rabbit D6-R8 on the two-hundredth day following the identical treatment given rabbits A5-9 and F4-7, lesions from which are shown in figures 8 and 9, respectively. There are moderate numbers of bacilli in the epithelioid cells and mononuclears.

FIG. 11. (Lower right.) Another portion of the local lesion of rabbit F4-7 shown in figure 9. Intact mononuclears contain numerous tubercle bacilli.



FIGS. 8–11

swarmed with bacilli. These cells, penetrating between the collagen bundles, caused necrosis of the latter *en masse*. Extension by the perivascular lymphatics was very prominent. They also teemed with bacilli. Occasionally a rabbit was found in this family, C4R-9 for example, in the epithelioid cells of which bacilli were rather few. It is interesting that this rabbit died on the eighty-ninth day, of an intercurrent disease, with only slight generalized tuberculosis; furthermore, the nodes draining its portal of entry were free from tuberculosis.

Thus again it is found that the F family is uniform in its response to tuberculosis. In the C family, however, while the majority respond in essentially the same manner as the F's, some rabbits exhibit considerable capacity to inhibit the growth of the bacilli within their cells.

The families of intermediate resistance, B, D and H, were also mixed in their response to the tubercle bacillus at the portal of entry. Thus 2 rabbits of the B family and one of the H family had completely healed their lesion at the portal of entry. None of the D family, however, healed its lesion completely. In the remaining rabbits of these families the local lesion was as a rule well encapsulated and either softening, liquefaction or calcification had overtaken the caseous center of the lesion at the portal of entry. Extension was usually by contiguity. Occasionally however the perivascular lymphatics became invaded by cells laden with bacilli and, at the periphery of the lesion, the epithelioid cells harbored numerous microörganisms (figure 10). In some rabbits the cellular debris insinuated itself between the collagen bundles and caused their necrosis. But in no rabbit of these families were the bacilli quite so numerous in the cells, the encapsulation so meagre and the perivascular lymphatics so crowded with bacilli as in the susceptible F or C family. Thus families B, D and H occupy an intermediate position in the capacity of their mononuclears to inhibit the growth of tubercle bacilli. Again it is found that the response of these families is not uniform, some sharing the characteristics of the susceptible families and others those of the resistant rabbits.

It is interesting that in this experiment the order of resistance in these three families was as follows: B, with an average survival of three hundred and two days, H with two hundred and sixty-six, and D with two hundred and four. Corresponding to this graded resistance was the degree of healing at the portal of entry in these three families. B headed the list; next came H and last the D family.

Thus an analysis of the lesion at the portal of entry in these six families

reveals that resistance is primarily a function of the acquired capacity of the mononuclears to inhibit the growth of the bacilli. It is also apparently influenced by the cohesiveness of their connective tissues and the propensity of their lymphatics to become transportation channels for the tubercle bacilli. The rapidity of the mobilization of their mononuclears and the rate of their maturation also seemingly influence the resistance of these rabbits.

A summary of this study as well as of the preceding two reports is given in the next paper of this series.

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IV. HEREDITARY CONSTITUTION AND TUBERCULOSIS SUMMARY AND DISCUSSION

CLASSIFICATION OF FAMILIES ACCORDING TO THEIR RESISTANCE

The tuberculosis resulting from various modes of infection in generation after generation of 6 rabbit families inbred by brother-sister or parent-offspring matings and maintained under uniform environmental conditions shows that resistance to the disease is a function of genetic constitution. These 6 families fell into three groups. The A family was uniformly highly resistant. The F family and the second inbred generation of the H family were uniformly highly susceptible. With few exceptions, the C family was likewise highly susceptible. The families B and D and the third and fourth generations of the H family were intermediate in their resistance to tuberculosis.

METHODS OF DETERMINING RESISTANCE

Resistance was determined by three independent methods of infection. (1) Statistically reliable differences in the survival time of these three groups of families could be demonstrated by inoculation intravenously or intracutaneously of appropriate standard doses of virulent bovine type tubercle bacilli. This relative resistance, however, could be overwhelmed by sufficiently large doses. (2) Definite differences in survival time between the resistant A family and the susceptible F family could be shown by causing them to inhale known small numbers of tubercle bacilli in a single exposure in a specially designed apparatus. It must be emphasized, however, that these differences could be abolished by the inhalation of large numbers of tubercle bacilli at one sitting. Furthermore, by this method, and with small numbers of bacilli, the disease produced closely simulated the widely differing types of tuberculosis acquired by these two families respectively on natural exposure to tuberculous cage mates (figures 1 and 2 of second paper). (3) The method of infection which elicited the fundamental differences between the families was that of natural respiratory contagion. In this method the members of a given generation of all the families were simultaneously exposed to artificially infected rabbits shedding tubercle bacilli in their urine and

placed on the other side of centrally situated screens in a special cage (text figure 1 of first paper). The cage was so constructed that the passage of air from the sources of contagion to the contacts was obligatory. Under these conditions of exposure, simulating the mode of infection in man, each of the three groups of rabbit families acquired a tuberculosis of pulmonary origin characteristic for each group, generation after inbred generation, having the essential nature of the various types of the disease acquired by different ethnic groups of human beings.

CHARACTER OF THE NATURALLY ACQUIRED RESPIRATORY DISEASE

On natural respiratory contagion the A family of the second, third, fourth and fifth generations acquired a tuberculosis of long duration, which usually originated in a single, slowly progressive, encapsulating primary focus that became excavated. The draining tracheobronchial nodes did not become involved either grossly or microscopically. The disease was confined chiefly to the portal of entry, the lungs, where it extended by canalicular spread from the softened primary lesion and where the secondary foci progressed in a similarly chronic manner until they became excavated in turn. There was little dissemination of the disease to the rest of the body by the blood or lymph (figure 1 of first paper). Even in the few instances of pulmonary haematogenous dissemination these tubercles did not prosper (figure 2 of first paper). The disease picture was usually dominated by a localized ulcerative pulmonary phthisis analogous to the reinfection type as seen in white adults. Microscopically the lesions in the A family consisted of large areas of mature, intact epithelioid cells in which tubercle bacilli were rarely found. It was only in caseous foci undergoing softening that the bacilli accumulated to any considerable extent (figure 17 of first paper). It was only through the aspiration of the bacillus-laden softened material into preformed channels that the disease extended. This obviously resulted from overwhelming the natural high resistance of the tissues by flooding them with vast numbers of microörganisms. Lesions of lymphogenous and haematogenous origin healed by resolution or cicatrization (figure 16 of first paper), apparently because of the relatively smaller number of bacilli that were focalized in different parts of the body by these routes. Even the foci of softening tended to become surrounded by dense layers of fibrous tissue (figure 14 of first paper), with intact epithelioid cells remaining at the edge of the softening mass. It seems reasonable to infer that the mononuclear phagocytes of the A family acquired a capacity

to inhibit effectively the growth of the tubercle bacillus in their cytoplasm.

By contrast, the second, third and fourth inbred generations of the F family developed a rapidly fatal disease. This again originated as a single primary focus in the lung, but it progressed rapidly to caseation and did not become encapsulated or excavated. Almost simultaneously the draining tracheobronchial nodes became massively enlarged and caseous. The tuberculosis then disseminated by the blood to many internal organs, where it caused large destructive caseous nodular lesions (figures 3 and 4 of first paper). Death followed before softening or canalicular spread could take place. The disease was a primary, rapidly progressing type of generalized tuberculosis. It simulated the so called childhood type of tuberculosis and resembled that seen by Borrel in the Senegalese troops. Equally sharp was the contrast in the microscopic picture between the A and the F family. In the primary focus of the F rabbits tubercle bacilli swarmed in the mononuclear cells that were still alive at the edge of the rapidly extending caseous mass (figure 15 of first paper). The blood vessels that became enmeshed in this rapidly advancing process underwent necrosis *en masse* and remained agape in the necrotic tissue teeming with tubercle bacilli (figure 18 of first paper). Conditions were ripe for blood invasion by the microorganism. Occasionally minute foci of softening developed. These gave rise to sequestrating microscopic cavities without fibrous tissue encapsulation, the ragged edges of which were formed by necrotic epithelioid cells lying in the adjacent normal alveoli and containing innumerable bacilli (figure 13 of first paper). The bacilli that invaded the lymph and blood vessels gave rise to similar progressive rapidly caseating lesions in the draining nodes and the internal viscera. Even the fibroblast-like cells of the abortive capsules of these secondary lesions swarmed with bacilli. It is clear that the mononuclears of the F family did not acquire any considerable capacity to inhibit the growth of the bacilli in their substance.

All the members of the second inbred generation of the H family behaved in essentially the same manner toward this tuberculous infection as did the F family (figure 7 of first paper). In fact they died even more rapidly than the members of the F family. Although the microscopic picture of their disease suggested certain characteristics for resistance, such as epithelioid cells with few bacilli, fibrous encapsulation and softening, these favorable factors were apparently overcome by a particular

vulnerability of the blood vessels to bacillary invasion. Tubercles formed in their medial coat and these gradually eroded the endothelium and shed their contents into the lumen. For this reason, apparently, these rabbits died even more rapidly than the members of the F family.

The great majority of rabbits of the C family of the second, third, and fourth inbred generations behaved in essentially the same manner toward tuberculosis as did the F family (figure 5 of first paper). In occasional rabbits, however, the individual lesions progressed slowly, although the disease as a whole was still a primary generalized tuberculosis (figure 6 of first paper).

The families B and D in the second, third, fourth and fifth inbred generations, and the third and fourth inbred generations of the H family showed an intermediate degree of resistance to tuberculosis. That is, some rabbits developed primary progressive generalized tuberculosis (figure 9 and 11 of first paper), and others slowly progressive tuberculosis of an intermediate type with slight to moderate dissemination to the draining nodes and internal organs (figures 8, 10 and 12 of first paper). A few developed the type of disease characteristic of reinfection with ulcerative phthisis, as seen in the A family. Similar microscopic differences were noted in these families. Their behavior was not uniform like that of the A or F rabbits. In some the cells behaved like those in the former family; in others their behavior was akin to that of the latter. Still, even the most resistant members of these families did not show quite the same degree of localizing and healing capacity that the members of the A family exhibited. On the other hand the most susceptible members of these families of intermediate resistance did not show quite the same fulminating progress and the same invasive characters that were uniformly found in the F and C families. The tuberculosis developed by these families resembled to some extent the types seen in the American Negro.

OTHER FACTORS STUDIED IN RELATION TO RESISTANCE

There was no conspicuous difference between the families in the time elapsing between the beginning of exposure and the onset of tuberculosis. Neither sex nor age, beyond three months, played a significant rôle in the resistance of these families to tuberculosis. In some families there was a correlation between the spread of India ink in the skin before exposure and susceptibility to tuberculosis. Thus the highly resistant family A restricted the spread of the dye, whereas the highly susceptible

family F permitted it to spread more widely. However, this correlation was not uniform. Again, the allergic sensitivity developed on natural infection by the susceptible family F was greater than that developed by the resistant family A. This was probably due to the greater multiplication of tubercle bacilli, and hence the greater stimulation that took place in the susceptible family. This may be compared with the fact that Negroes exhibit higher sensitivity to tuberculin than the white population. However, this relationship did not hold in all families.

Breed and color markings were not significant factors in resistance to tuberculosis. Families A and B were of the same breed and had the same English distribution of colors; yet their resistance differed conconspicuously. Furthermore, color markings may be the same and yet the breed may be quite different. Thus English-Dutch heterozygotes are indistinguishable from the homozygous dominant English variety. The two families differing most sharply and constantly in their resistance, A and F, often had the same colors. The second inbred generation of the H family and the third and fourth generations of the same family, were all of the same low Dutch variety of color markings; yet these generations differed widely in their resistance. It appears to be the total genetic constitution of the rabbit, apart from its breed or color, which determines its resistance to tuberculosis.

ALLERGIC IRRITABILITY, ANTIBODY PRODUCTION, PERMEABILITY AND RESISTANCE TO TUBERCULOSIS

In an attempt to shed further light on the nature of the determinants for resistance to tuberculosis, the studies recorded in the preceding paper were undertaken. It was found that the resistant family A responded to treatment with heat-killed tubercle bacilli, a non-multiplying antigen, by developing allergic sensitivity rapidly and to a high and constant level. The family of greatest susceptibility, F, developed sensitivity very slowly and to a lower level. Family B of intermediate resistance developed sensitivity at an intermediate rate and to an intermediate degree. This relationship, however, did not hold strictly in all families. Similarly, the highly resistant A family developed agglutinins to tubercle bacilli rapidly and to a high titre. Susceptible family F produced these antibodies much more slowly and to a lower titre. Family D of intermediate resistance developed these antibodies at an intermediate rate and to an intermediate degree. Again, however, the capacity for antibody production did not always parallel resistance to tuberculosis.

There seemed to be an inverse relationship between the spread of India ink in the skin of a family after a uniform tuberculous infection and the survival of that family. Thus families A, F and C spread the dye over the following areas on the sixtieth day of infection: 390, 934 and 1570 sq. mm. respectively. The most resistant family spread the least; the most susceptible families, F and C, spread the most. However families F and D, which differed greatly in their resistance, did not differ in their capacity to spread the dye.

THE DEVELOPMENT OF LOCAL IMMUNITY AND RESISTANCE TO TUBERCULOSIS

Up to this point no single behavior pattern presented has appeared uniformly associated with resistance to tuberculosis. However, one fundamental factor was intimately and constantly correlated with resistance to the disease. The rate and intensity of development of local immunity, as indicated by the rate and effectiveness of healing of the lesion at the portal of entry in a cutaneous infecton, invariably yielded an index of the ultimate fate of a given rabbit family. This could best be demonstrated by subjecting the different families to preliminary treatment with heat-killed tubercle bacilli. Under these conditions, the most resistant family, A, developed lesions which reached their peak in the shortest time. Thereafter healing was rapid and complete in the majority of instances (figure 5, paper 3). Late in the course of the disease slight exacerbation of these foci occurred. The rabbits survived for a long time, dying finally from chronic ulcerative pulmonary phthisis. In the most susceptible families, F and C, the lesion grew apace for a much longer time. Healing set in tardily (figure 3, paper 3) and was only abortive. Severe exacerbation of the lesion soon occurred and the animals died shortly of generalized, rapidly progressive tuberculosis. The families of intermediate resistance showed an intermediate rapidity of healing (figure 4, paper 3) and an intermediate degree of exacerbation. They died of a disease of intermediate duration, which had the character of a chronic slowly progressive generalized tuberculosis. Essentially similar though less pronounced differences in the skin lesions of the susceptible and resistant families have been obtained by the intracutaneous inoculation of the same strain of virulent bovine bacilli without preliminary immunization (figures 1 and 2, paper 3).

Microscopic examination of the lesions at the portal of entry showed that the epithelioid cells mobilized in the A family effectively destroyed the tubercle bacilli (figure 8, paper 3). The latter multiplied only in

thoroughly encapsulated foci undergoing softening. Lymphatic invasion was inconspicuous. In the susceptible families the mononuclears swarmed with tubercle bacilli (figure 11, paper 3). There was massive caseation and little encapsulation, and the dissemination of bacilli by way of the lymphatics was phenomenal (figure 9, paper 3). In the families of intermediate resistance the response at the portal of entry was mixed. But, in general, their mononuclears contained fewer bacilli, encapsulation was more pronounced, and lymphatic dissemination was less marked than in the susceptible families (figure 10, paper 3). Furthermore, the nodes draining the portal of entry in the skin of the resistant family A underwent an abortive tuberculosis which healed. In the susceptible families the nodes were greatly involved and caseous. In the families of intermediate resistance softening overtook these lymph nodes.

LOCAL IMMUNITY AND THE NATURALLY ACQUIRED RESPIRATORY DISEASE

A parallel has been established between the behavior of the primary focus of the naturally acquired infection in the lungs of these families of varying resistance, and the lesions at the site of a skin inoculation in the same families previously immunized by treatment with heat-killed tubercle bacilli. The differences observed are primarily expressions of differences in anatomical structure in these two portals of entry. Canalicular spread takes place in the lung. No such avenues for dissemination are found in the skin. In the resistant families, in the naturally acquired tuberculosis of pulmonary origin, the disease extended slowly by tubular spread in the lungs, while the lesion resulting from skin inoculation healed completely in the majority of instances. In the susceptible families the lesion at the portal of entry extended fulminatingly both in the lung and in the skin. In the families of intermediate resistance the lesions were mixed in their character in both sites, healing in some individuals and extending in others; but in neither of these portals of entry did the lesions quite approximate the character of the most resistant family on the one hand, or that of the susceptible families on the other.

Likewise parallel was the dissemination of the disease from these two portals of entry in the various families. In the resistant family, at death, the lymph nodes draining both portals of entry were usually free from tuberculosis. In the susceptible families they were massively caseous in both. In the families of intermediate resistance these nodes were variable in the degree of involvement in both infections.

The intimate microscopic picture in these two diseases showed the same general tendencies. The resistance of these families was ultimately associated with the capacity of their mononuclears to acquire increased power to destroy or inhibit the growth of the bacilli. The rate at which this was acquired and its intensity at the portal of entry primarily, but also in the body generally, influenced the outcome of the disease in the various families. Thus it would appear that the character of the primary focus in the natural infection is essentially an expression of the rate and intensity of development of local immunity.

FAMILY GENETIC HISTORY, GENERAL RESISTANCE AND THE DEVELOPMENT OF LOCAL IMMUNITY

The intimacy of the correlation between the rate and intensity of development of local immunity and the inherited resistance of these families is thus demonstrated by the complete parallel of their behavior in the different families. Furthermore, analysis of the family trees of the rabbit groups suggests that both resistance and the character of the local immunity developed were functions of the genetic constitution of their forbears. Thus the families A and B shared a common ancestor (Charts 1 and 2 of first paper) and the B family was closest to the A both in its general resistance and in the efficacy of its development of local immunity. The F family, highly and uniformly susceptible to the infection, and developing uniformly a slow and feeble local immunity, was almost solely the product of repeated inbreeding of two rabbits, 1 and 27 (chart 6 of first paper). The C family, the preponderant ancestors of which were again the same two rabbits (chart 3 of first paper), was also, in the majority of instances, equally susceptible and equally ineffective in the development of its local immunity. In the immediate parentage of the H family the same two rabbits figured again (chart 8 of first paper). It is not surprising, therefore, that this generation's behavior resembled that of the F and C families. However, among the forefathers of their parents were some rabbits that entered into the ancestry of the D family (chart 4 of first paper) which is one of intermediate resistance generally and one which developed an intermediate degree of local immunity. The latter and other rabbits far outnumbered rabbits 1 and 27. It is understandable, therefore, that in the later inbred generations of the H family the constitution of the remaining ancestors should appear and that these generations should be variable in their response to infection and of a relative general resistance and a

degree of development of local immunity similar to that of the D family, some of whose ancestors they shared. In brief, families of similar general resistance and similar character of development of local immunity shared some common ancestors. Furthermore, the degree of uniformity of development of local immunity and general resistance of a family was a function of the degree of inbreeding of its forbears.

CONTRASTING CHARACTERS IN THE TWO MOST CONTRASTING FAMILIES

In the two most sharply and uniformly differing families, the highly resistant family A and the highly susceptible family F, the following contrasting factors obtained: The permeability of the skin of the A family was low (figure 7, paper 3); in the F family it was high (figure 6, paper 3). The development of allergic sensitivity in response to heat-killed tubercle bacilli was rapid and of great intensity in the A family; it was slow and of low degree in the F family. On the contrary, the allergic sensitivity developed during the course of a virulent natural infection was high in the F family and low in the A family. The antibody production on stimulation with killed tubercle bacilli was rapid and of high titre in the A family; it was slow and of low titre in the F family. The development of local immunity was rapid and intense in the A family; it was slow and feeble in the F family. The capacity of the mononuclears of the A family to destroy or inhibit the growth of tubercle bacilli in their cytoplasm was intense; it was slight in the mononuclears of the F family.

Incomplete data suggest that in response to heat-killed tubercle bacilli the mononuclears of the A family were rapidly mobilized and matured quickly; in the F family they were mobilized more slowly and matured tardily. Phagocytosis of tubercle bacilli was apparently more active in the A family and less in the F family. The cohesiveness of the connective tissue of the A family was considerable; it was apparently poor in the F family.

The relative resistance of the remaining families was definitely correlated with the relative capacity of their tissues to acquire a local immunity. This in turn, was correlated with the propensity of their mononuclears to acquire inhibitory properties against the multiplication of the bacilli in their cytoplasm. However, the relative permeability of their skin, the rapidity and intensity of their allergic responsiveness and the rapidity and intensity of their antibody production were not always graded in accordance with their graded resistance, although in relation to all of these qualities one or another of the families of intermediate

resistance fell in an intermediate position. It is apparent that they carried determinants for both susceptibility and resistance in varying combinations. Hence their intermediate position in resistance and the variable composition of constitutional factors associated with resistance and susceptibility. Lewis and Loomis (1), in their studies on the inherited resistance of inbred guinea pig families, also came to the conclusion that resistance to tuberculosis is controlled by numerous determinants.

The greater permeability of the skin of the highly and uniformly susceptible family F, generation after inbred generation, as compared with that of the equally uniformly higher resistant family A tempts some speculation. It is clear that all the differences noted between these families can be considered as expressions of the relative rapidity and intensity of the response of the cells to stimulation by tubercle bacilli. This different response may be an innate and ultimate property of the tissues concerned. It may also, however, be a result of different conditions acting upon cells of ultimate uniform capacities. It is axiomatic in biology that the response of protoplasm is proportional, within certain limits, to the intensity of the stimulus. The more concentrated the irritant the greater the response. Is it not conceivable that the greater capacity of the tissues to become sensitized to the tubercle bacillus, their greater antibody response and their more rapid acquisition of properties effectively to inhibit the growth of bacilli, etc., were all dependent upon the lower permeability or greater cohesiveness of the connective tissues of the more resistant family? Is it not conceivable that as a result of the lower permeability of the tissues in the resistant animal the irritant was more concentrated within a given area of receptive tissue and hence the response of the cells of the more resistant animal was more rapid and more intense?

A similar concept has been urged by Sabin (2) in explanation of the more effective skin sensitization that results from intracutaneous as compared with intraperitoneal or intravenous administration of an antigen. She presumes that the close felt work of the cutis restricts the antigen to a small zone and thus affords a "greater dose per cell" than occurs when the stimulating agent is widely dispersed over the entire peritoneal cavity or throughout the body by the blood. Was it this effect of varying permeability on cells of equal capacities in the different families, or must we resort to the hypothesis that the cells themselves in the susceptible and resistant families differed in their primordial

responsiveness to a given irritant? These are challenging questions which invite further investigation.

IMPLICATIONS OF STUDY FOR HUMAN TUBERCULOSIS

When the data presented in this study on rabbits are applied to the problem of pathogenesis of tuberculosis in man certain remarks are pertinent. It is clear that if resistance to tuberculosis in man is controlled by determinants similar to those active in rabbits, genetic constitution will determine whether an adult will acquire a disease of progressive primary or phthisical character. If the constitution is composed of factors for resistance the individual will acquire on first infection, without previous contact with the disease, a localized ulcerative phthisis, without involvement of the draining nodes and internal viscera, a tuberculosis anatomically of the "reinfection type." If his genetic constitution is made up of factors determining susceptibility he will acquire the primary, rapidly progressive type. He may acquire this latter type even in the presence of a primary infection from an earlier period. Hence, on this hypothesis, the rapidly progressive and destructive tuberculosis seen in the Negroes working in the mines of the Transvaal in spite of the fact that their disease is usually a reinfection. It has been demonstrated in this study that the acquired immunity is superimposed on the natural resistance. A highly susceptible animal cannot be immunized to any considerable degree, whereas a highly resistant animal can be immunized, even with heat-killed tubercle bacilli. In a majority of rabbits of the highly resistant family A so treated, the skin lesion resulting from the intracutaneous inoculation of 0.2 mg. of highly virulent tubercle bacilli, containing about 100,000,000 microörganisms, healed completely. The draining nodes underwent an ephemeral involvement which soon healed. If the individual in question has determinants both for resistance and susceptibility the type of tuberculosis developed will depend upon the varying combinations of these factors. However, it is clearly possible that a primary infection in an individual of intermediate resistance will determine the development of a reinfection type on later exposure. For in certain individuals, belonging to families of intermediate resistance, the site of virulent infection of the skin following a preliminary treatment with heat-killed tubercle bacilli also healed completely.

The type of disease developed by the several rabbit families of different genetic constitution, under uniform environmental conditions, and ex-

posed to tuberculosis in a manner simulating closely that obtaining in human life, was of essentially the same varying nature as that found in different ethnic groups. The localized, ulcerative, pulmonary phthisis characteristic of white adults was acquired by the most resistant A family. Primary, rapidly progressive, generalized tuberculosis, as found in the Senegalese troops, was characteristic of the highly susceptible families F and C. The mixed type of tuberculosis, intermediate between these extremes, as found in the American Negro, was typical of the tuberculosis developed by the families of intermediate resistance B, D and H. It is likely, therefore, that the varying susceptibility to tuberculosis of the different human groups depends to a large extent on varying genetic rather than environmental factors.

The work of Israel and Long (3) on adolescents with recent primary infection showed that a variety of anatomical and clinical forms obtained. In some patients a disease roentgenologically and clinically typical of primary infection occurred. In others tuberculosis of the type characteristic of reinfection developed. It is noteworthy that the former type was much more frequently seen in the Negro. However, the parallel between these human patients and the rabbit families here described was incomplete. For the fate of the lesion in the adolescents was not necessarily determined by its anatomical type as was the case in our rabbits. Rapidly progressive, rapidly retrogressive or torpid forms of clinical tuberculosis resulted in these cases irrespective of the anatomical nature of the pulmonary lesion as roentgenologically assessed. Whether these differences are due to factors inherent in the two species or whether they are the result of unknown variables such as intensity of exposure, endocrinological maturation and other differences, is uncertain.

The fact that the primary pulmonary lesion in all cases tends to be single, both in man and rabbits (figure 4, paper 2), suggests that comparable quantities of tubercle bacilli are involved in both instances. If, however, rabbits are caused to inhale known small numbers of units of tubercle bacilli, not one primary focus results, but a number, in proportion to the units of tubercle bacilli inhaled (figure 3, paper 2). This suggests that in human tuberculosis as well as in tuberculosis acquired by rabbits in natural respiratory contagion, about one unit is involved. Of how many bacilli this unit must be composed, when trapped in the terminal alveoli, to cause a primary pulmonary lesion nothing is known. It is hoped that with our rabbit families of known resistance to tuber-

culosis and with an apparatus refined and adjusted for accurate measurements of minute numbers of tubercle bacilli inhaled, these questions may be answered.

CONCLUSIONS

1. The resistance of rabbits to naturally or artificially acquired tuberculosis is a function of their genetic constitution.

2. The inherited resistance of rabbits to artificial infection may be overwhelmed by large doses of tubercle bacilli.

3. The genetic constitution *per se* determines whether, under given conditions of natural respiratory contagion, rabbits will acquire (a) a rapidly progressive primary generalized tuberculosis, (b) a localized chronic ulcerative pulmonary phthisis analogous to the reinfection type or (c) a disease of a character intermediate between these extremes.

4. The disease types acquired by rabbit families of varying inherited resistance in the investigation here reported were essentially analogous to the different types of tuberculosis developed by different human ethnic groups.

5. The fundamental variant in the disease developed by these families was the degree of localization of the infection at the portal of entry.

6. This localization of the infection is controlled by the chronicity of the disease at the portal of entry. In the lung the primary focus in the resistant family studied progressed slowly, became encapsulated and underwent excavation. Dissemination took place chiefly by tubular spread of the softened material in which alone the bacilli multiplied.

7. The mononuclears of the resistant family acquired an effective capacity to inhibit the growth of the bacilli, which prevented their multiplication in organs focalized by lymphogenous or haematogenous routes.

8. The mononuclears of the susceptible families studied did not acquire any considerable capacity to interfere with the growth of the bacilli in their cytoplasm. As a result, the primary focus progressed fulminatingly and dissemination and multiplication of the bacilli took place in the organs focalized by lymphogenous and haematogenous routes.

9. Certain families of intermediate resistance varied in the capacity of their cells to acquire inhibitory properties against the multiplication of bacilli in their cytoplasm. Some behaved like the resistant rabbits; others resembled the susceptible animals. None of them, however, acquired this property to the same degree as the former or failed to do so equally with the latter.

10. Resistance to tuberculosis was intimately associated with the intensity and rapidity with which the tissues of the different families acquired a local immunity. The latter developed most rapidly and reached the highest degree in the most resistant family; it was slowest in development and feeble in the most susceptible families; in the families of intermediate resistance it was variable and usually intermediate in rapidity and intensity.

11. The progress of the primary pulmonary lesion and the dissemination of the disease therefrom in the different families were functions of the rapidity and intensity of development of this local immunity.

12. In addition to the above named factors, in the two most sharply and uniformly contrasting families, low skin permeability, rapid and intense development of allergic sensitivity and rapid and high production of antibodies were associated with resistance; high permeability, slow and feeble development of allergic sensitivity and tardy and low production of antibodies were associated with susceptibility.

13. In the remaining less uniformly behaving families some possessed these qualities in a degree corresponding to their resistance; others did not. This was associated with the heterogenicity of their constitution.

14. It is the sum total of numerous determinants that controls hereditary resistance to tuberculosis.

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